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Estudio games de endocarditis infecciosa en España

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

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ABBREVIATIONS

IE: infective endocarditis.

EHS: Euro Heart Survey.

VHD: valvular heart disease.

E-BSI: Enterococcus bloodstream infection.

TEE: transesophageal echocardiography.

TTE: transthoracic echocardiography.

CT: computed tomography.

MRI: magnetic resonance imaging

18F-FDG: 2-[18F]-fluoro-2-deoxy-D-glucose.

PET: positron emission tomography.

GAMES: Spanish Collaboration on Endocarditis-Grupo de Apoyo al Manejo de la Endocarditis Infecciosa en España

IVDU: intra venous drug user.

OR: odds ratio.

CI: confidence interval.

SD: standar deviation.

IQR: interquartile range.

BC: blood cultures;

CNS: coagulase-negative staphylococci.

MRSA: methicillin-resistant *S. aureus.*

CNS: central nervous system.

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SUMMARY

INTRODUCTION

Infective Endocarditis History Review

The very term "endocarditis", referring to an individual tissue and an inflammatory process, goes back to early-nineteenth-century with clinicians such as Broussais and Bouillaud, [1]. However, it was very difficult during that period to define endocarditis in a simple, unequivocal manner[2]. Thus, the term "endocarditis" continued to be used for a disease which underwent endless development throughout the nineteenth century. William Bart Osler (1849-1919) is the eponym linked to infective endocarditis in general (Osler's disease) and one of its peripheral manifestations (Osler's nodes). He established that blood elements such as fibrin and platelets deposited on the damaged endocardium - substrate of nonbacterial thrombotic endocardiopathy - and constituted the nucleus of vegetation, devaluing the concept that it depended on secretions from the endocardium [6].

Stimulated by Osler's presentations, Lord Thomas Jeeves Horder (1871-1955)[5], emphasized the pre-existence of valvulopathy and congenital cardiopathy, the importance of the oral and intestinal points of entry, the occurrence of mycotic aneurysm, the presence of splenomegaly and the identification of streptococcal etiology in more than 60% of the cases confirmed through necropsies [7]. Horder recognized five types of infective

endocarditis: 1 - latent; 2- fulminant; 3- acute; 4- chronic and 5- subacute, a modality that corresponded to 70% of the cases.

The 20th Century and the introduction of antibiotics

It was no after penicillin availability became universal, thanks to the pioneer efforts of Englishmen such as Nobel-prize winner Howard Florey (1898-1968) and Americans such as Martin Henry Dawson (1896-1945), that the treatment of infective endocarditis started an efficiency ascension [8]. In the 1960s, it was established that infective endocarditis was a curable disease. Andrew G. Wallace *et al.* [9] at Duke University, understood that the removal of the infected valve and its substitution by a valve prosthesis constituted an approach on the clinical manifestation cause. The routine surgical indication in cases of infective endocarditis in cases with CHF grade III/IV (the most common recommendation) reduced mortality from 90% to 10%.

Introduction of imaging techniques, in the 1950s into the infective endocarditis field, represented a remarkable evolution in the support of the diagnostic rationale of infective endocarditis by joining the traditional clinical data and images and calculations determined by technological advancement in echocardiography.

In the 1990s, David Durack et al. [10], from Duke University gave another magnificent contribution to the knowledge of infective endocarditis, through a diagnostic systematization that elevated echocardiography as a determinant method of a major criterion.

In 2000, Jennifer S. Li, a professor of Pediatrics from Duke University and colleagues [11] presented a perfected version of the 1994 criteria from Duke University; completing the outstanding advancement in the frontier of knowledge on infective endocarditis in the 20th century.

GENERAL BACKGROUND

The term infective endocarditis (IE) denotes infection of the endocardial surface of the heart and implies the physical presence of microorganisms in the lesion [12]. There have been several classifications for IE. Classically, the distinction was based on progression of the untreated diseases (acute – subacute – chronic)[16], but since the introduction of antibiotic treatment a classification based on the etiologic agent and/or clinical setting (native – prosthetic - device) is preferable [17].

IE is a severe disease with very significant morbidity. Data on mortality vary widely, ranging from 13% to 25% during admission, with increases of 9% to 20% within one year after discharge [18-22].

EPIDEMIOLOGY

They are no population studies on EI in Spain, so it is not possible to determine the exact incidence of this disease in our country; but large tertiary care institution studies [21] have reported an estimated incidence of 6.4 cases per 100,000 inhabitants per year, 0.8 cases per 1,000 admissions and 3.5% of all episodes of significant bacteremia.

Among hospital-based studies, IE epidemiology changed mainly in three aspects over the last 5 decades [20]. First, patients are significantly older (1980s: mean age 45.3, CI 40.2- 50.5 vs 2000s: mean age 57.2, CI 54.7- 59.7, $p<0.001$). Second, more are men (1970s: 58.6%, CI 54.3- 63.0 vs 2000s: 66.3%, CI 63.6- 69.0, $p<0.01$); and third, the percentage of IE cases occurring on prosthetic valves increased over time (1960s: 8.4%, CI -3.8- 20.5 vs 2000s: 22.9%, CI 19.1 - 26.7, $p=0.05$).

MICROBIOLOGY

A great variety of microorganisms can cause infective endocarditis (IE); there have been significant changes in microbiology over time; *Streptococcus viridans* IE markedly decreased in percentage over time meanwhile, frequency distribution of other microorganism like *Staphylococcus aureus* IE and coagulase-negative *Staphylococcus* IE have increased. More recently, culture negative IE decreased while enterococcal IE percentage increased significantly over the last decade.

Enterococcal IE

The enterococci are Gram-positive, facultatively anaerobic oval cocci that form chains of various lengths; all enterococci are in the Lancefield group D; they are catalase-negative and non-motile with a homofermentative metabolism. These microorganisms are usually able to grow at temperatures ranging from 10 to 45°C with optimum growth at 35 to 37°C [31].

The enterococci are commensal microorganisms that act as opportunistic agents causing a variety of infections in humans. *Enterococcus faecalis* is the most common human pathogen, but *Enterococcus faecium* has become increasingly prevalent in hospital-acquired infections. All the other enterococcal species together constitute less than 5% of enterococcal infections [39, 40]. The enterococci most commonly infect the urinary tract, bloodstream, endocardium, burn and surgical site wounds, abdomen, biliary tract, and catheters and other implanted medical devices [16, 33].

Over the last decades, they have emerged from being considered virtually harmless bacteria to medically important multiple-antibiotic-resistant health care-associated pathogens that contribute significantly to patient morbidity and mortality as well as health care costs [28].

The ability to form biofilms has recently been listed among the most prominent virulence properties of these microorganisms, allowing colonization of inert and biological surfaces while protecting against antimicrobial substances and mediating adhesion and invasion of host cells [46]. Biofilm formation may be of particular importance in the development of endocarditis, as well as implant and other medical-device associated infections [28, 47, 48]. in Spain [51], up to one-third of infective endocarditis patients become infected through contact with the health care settings.

E. faecalis remains the more common cause of enterococcal endocarditis. These heart valve infections typically occur in older patients [55, 57, 58]. The initial source of bacteremia leading to endocarditis is usually the genitourinary or gastrointestinal (GI) tract. A recent large-case series of enterococcal endocarditis reported that between 15% and 39% are healthcare-associated [55, 57].

The clinical picture of enterococcal endocarditis is usually one of subacute infection characterized by heart failure, rather than embolic events [57]; however, rapidly progressive disease can also occur. Establishing an early diagnosis of enterococcal IE it is essential to improve the outcome of these patients.

DIAGNOSIS

The variability in clinical presentation of IE requires a diagnostic strategy that is both sensitive for disease detection and specific for its exclusion across all forms of the disease. In 1994, Durack and colleagues, [10] from Duke University Medical Center proposed a diagnostic schema termed the Duke criteria, which stratified patients with suspected IE into 3 categories: “definite”, “possible” and “rejected” cases.

The Duke criteria incorporated echocardiographic findings in the diagnostic strategy. Six common but less specific findings of IE also were included as minor criteria in the original Duke schema: intermittent bacteremia or fungemia, fever, major embolic events, non-embolic vascular phenomena, underlying valvular disease or injection drug use, and echocardiographic abnormalities that did not full fill the typical diagnosis of valvular vegetations, abscesses, or dehiscence.

Therefore, IE it is diagnosed using a combination of clinical, microbiological, and imaging criteria [10, 12]. Failure to identify metastatic complications may lead to early interruption of therapy, thus triggering relapse and unfavorable outcome. Infectious embolisms can be asymptomatic and difficult to recognize [61], with the result that systematic performance of multiple imaging techniques (computed tomography [CT], magnetic resonance imaging [MRI], and ultrasonography)

has been recommended for all patients with IE[62]. However, this approach is time-consuming and cumbersome and involves frequent transfer of a very ill patient to the radiology department.

2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) positron emission tomography (PET)–CT has a promising role in infectious diseases, owing to its high sensitivity, anatomical precision, and lack of toxicity [64, 65]. The possibility of scanning the whole body with a single test is particularly appealing for clinicians treating patients with IE.

Studies analyzing PET-CT for the evaluation of patients with IE are scarce, lack a control group, evaluate a small number of patients, or consist of case reports [66, 67]. The field of examination in the recent report by Saby et al[68] was limited to the heart, and a high incidence of false-negative results were detected (33%).

OBJECTIVES

I. To assess the current situation of IE in a whole country.

1.1 To describe the changes in epidemiology and clinical manifestations of IE in a nationwide study.

1.2 To evaluate the risk factors of early and late mortality of IE during the first five years of the study.

II. To evaluate the epidemiological, microbiological, and clinical characteristics of Enterococcal IE

2.1 To identify characteristics that could help to identify IE among patients with E-BSI

2.2 To compared the outcome of E-BSI in patients with and without IE in order to assess the potential consequences of misdiagnosis.

2.3 To validate the NOVA score as a model for predicting patients with enterococcal bloodstream infection at low risk for IE.

III. To evaluate the systematic performance of new diagnostic imaging techniques in IE patients.

3.1 To evaluate the clinical impact of systematic whole-body 18F-FDG PET-CT (PET-CT) for the diagnosis of septic embolisms in patients with IE.

MATERIAL, METHODS AND RESULTS

I. Epidemiology and outcome of infective endocarditis in Spain.

Since January 2008, multidisciplinary teams have prospectively collected all consecutive cases of IE diagnosed according to the Duke criteria in 25 hospitals. Overall, 1804 patients were recruited from 2008 to 2012 by GAMES. The median age was 69 years (IQR, 55-77), 68.0% were men, and 37.1% of the cases were nosocomial or health care–related. Gram-positive microorganisms accounted for 78.8% of the episodes, followed by Gram-negative (5.2%), fungi (2.4%), anaerobes (1.2%), polymicrobial infections (1.9%), and unknown etiology (9.1%). Heart surgery was performed in 44.2%, and in-hospital mortality was 28.8%. Overall, 1035 (80.6%) surviving patients had a one-year follow-up, 69 needed heart surgery (6.6%), 32 relapsed (3.1%), and 116 died (11.2%; IE-related in 31-8%). The one-year independent risk factors for mortality were increasing age (OR, 1.02), neoplasia (OR, 2.46), renal insufficiency (OR, 1.59), and heart failure (OR, 4.42). Surgery was the only intervention that had a major impact on one-year mortality (OR, 0.44).

II. Risk factors and outcome of Enterococcus infective endocarditis.

Between September 2003 and October 2012, we performed a prospective cohort study (all patients with E-BSI) and a case-control study (patients with/without enterococcal IE) in our tertiary center.

We detected 1515 patients with E-BSI and 65 with enterococcal IE (4.29% of all episodes of E-BSI, 16.7% of patients with E-BSI who underwent transthoracic echocardiography, and 35.5% of all patients with E-BSI who underwent TEE). We developed a bedside predictive score for enterococcal IE—NOVA score—based on the following variables: Number of positive blood cultures (3/3 blood cultures or the majority if more than 3), 5 points; unknown Origin of bacteremia, 4 points; prior heart Valve disease, 2 points; Auscultation of a heart murmur, 1 point (ROC=0.83). The best cutoff corresponded to a score ≥4 (sensitivity, 100%; specificity, 29%).

A score <4 points suggested a very low risk for enterococcal IE and that TEE could be obviated. Depending on local prevalence of endocarditis, application of the NOVA score may safely obviate echocardiography in 14-27% of patients with enterococcal bacteremia.

III. Clinical impact of PET-CT on the diagnosis of septic embolisms in patients with infective endocarditis.

We performed a prospective cohort study (47 definite IE and PET-CT) with matched controls (94 definite IE patients not undergoing PET-CT) from January 2012 to July 2013 in a tertiary hospital. The results were compared with those of conventional diagnostic techniques and clinical follow-up. PET-CT revealed at least 1 lesion in 35 patients (74.5%): 18 showed an embolic complication, 8 a pathologic uptake on the valves or cardiac devices, 1 patient showed both, 5 had incidental non-infectious findings and 3 were considered false positives.

The validity values for the efficacy of PET-CT in diagnosis of septic lesions were as follows: sensitivity, 100%; specificity, 80%; positive predictive value, 90%; and negative predictive value, 100%.PET-CT was the only initial positive imaging technique in 15 true-positive cases (55.5%).

The systematic use of PET-CT was associated with a 2-fold reduction in the number of relapses (9.6 % vs. 4.2% p = 0.25) and enabled significantly more infectious complications to be diagnosed (18% vs. 57.4%; p = 0.0001).

CONCLUSIONS

I. IE remains an infrequent but severe disease that commonly presents in older patients with multiple underlying conditions and is frequently health care–related.

II. Multidisciplinary groups are essential to optimize the management and outcome of this IE; so far, the only intervention that has shown a major impact on one year mortality was surgery.

III. The prevalence of enterococcal IE depends on whether the sample comprised all cases among those with E-BSI (4.3%), only patients undergoing echocardiography (16.7%), or only patients undergoing TEE (35.5%).

IV. Depending on the local prevalence of endocarditis, application of the NOVA bedside prediction score could safely obviate echocardiography in 14-27% of patients with enterococcal bacteremia.

V. PET-CT is an effective way of accomplishing the extension study in a single test in patients with IE. It is easily performed (<2 h) and comfortable for the patient and provides the clinician with whole-body data.

VI. PET-CT enables significantly more infectious complications to be diagnosed (18.0% vs. 57.4%; p = 0.0001) and its use procured a trend toward a reduced number of relapses (9.6 % vs. 4.2% p = 0.25) in patients with IE.

RESUMEN

INTRODUCCIÓN

Revisión Histórica de la Endocarditis Infecciosa

El término "endocarditis", se refiere a un tejido individual y a un proceso inflamatorio, que retrocede a la primera parte del siglo diecinueve, con médicos como Broussais y Bouillaud, antes de que surgiera la teoría de los gérmenes y el nacimiento de la bacteriología [1]. Sin embargo, fue muy difícil durante este período definir la endocarditis de forma simple e inequívoca. No siempre existió una clara relación entre las ideas y los argumentos eran confusos, tortuosos, circulares y no concluyentes.

En su discusión sobre la sífilis, Ludwik Fleck, señaló que la enfermedad es un fenómeno cambiante que constantemente integra nueva información y conceptos [2]. Por lo tanto, el término "endocarditis" continúo siendo utilizado para definir una enfermedad que atravesó un largo proceso de desarrollo durante el siglo diecinueve. Simultáneamente, también existieron modificaciones en los vínculos etiológicos entre las anomalías anatómicas, los síntomas clínicos y las observaciones durante la autopsia. La patología de la enfermedad fue regularmente reformulada y su definición cambió de periodo a periodo y de país en país. La teoría de los gérmenes y el uso del microscopio cambio la visión y el concepto de la enfermedad a finales de siglo. El hecho de ser posible "ver" diminutas granulaciones hasta entonces invisibles no eran suficientes para

transformar estas granulaciones en una herramienta analítica. La percepción de que la enfermedad es un todo relativamente coherente con los síntomas etiológicos, llevaron sin embargo a la realización de experimentos en el laboratorio [3].

Hugo Ribbert (1855-1920) realizó experimentos sobre la inducción de endocarditis infecciosa, inyectando *Staphlylococcus aureus* cultivado en patatas, en conejos e indentifico colonias bacterianas sobre la superficie de las válvulas cardíacas, especialmente en la *chordae tendineae* de la válvula mitral. En el mismo año, WK Wyssokowitsch (1854-1912), obtuvo la colonización de bacterias en la válvula aortica, inyectadas en el torrente sanguíneo de conejos a través de la arteria carótida.

Gracias al conjunto de experimentos realizados en esa época, se obtuvieron dos conclusiones: a) la anterioridad de una endocardiopatía trombótica no bacteriana; b) la colonización del sustrato por bacterias circulantes. El siglo diecinueve termina con la asociación entre la lesión valvular, el punto de entrada y la circulación de microorganismos, la fiebre y las manifestaciones extra-cardíacas como la síntesis del diagnóstico de la endocarditis infecciosa [1, 4].

Al principio del siglo veinte, antes de que se vislumbraran los primero rayos de la esperanza terapéutica, algunos clínicos intentaron arrojar alguna luz, en el hasta entonces confuso conocimiento de la endocarditis y

superar el formidable problema de la descripción y clasificación de esta compleja enfermedad [5].

William Bart Osler (1849-1919), es el epónimo general, ligado a la endocarditis infecciosa (enfermedad de Osler) y una de sus manifestaciones perifericas lleva su nombre (nódulos de Osler). Osler percivió que existían casos simples y otros más complejos, y por lo tanto, el uso de términos discriminativos como ulcerativo, maligno, séptico y piógeno [6].

Osler estableció que los elementos sanguíneos, como la fibrina y las plaquetas se depositaban en el endocardio dañado - sustrato de la endocardiopatia trombótica no bacteriana - y constituían el núcleo de la vegetación, devaluando el concepto de que la lesión dependía de las secreciones del endocardio. Osler llamó la atención hacia la diversidad de microorganismos involucrados en la formación de vegetaciones y recopiló información a favor de la característica primaria en cuanto a la presencia de gérmenes en la etiopatogenia de la endocarditis infecciosa, en una época en la que la detección de microorganismos vivos en los cultivos sanguíneos era incipiente [6].

Simultáneo a la presentación de Osler, Lord Thomas Jeeves Horder (1871-1955) enfatizó la presencia de la valvulopatía y la cardiopatía congénita, la importancia de los puntos de entrada orales e intestinales, la presencia de

aneurismas micóticos, la presencia de esplenomegalia y la identificación de la etiología estreptocócica en más del 60% de los casos confirmados a través de necropsias [7]. Horder reconoció cinco tipos de endocarditis infecciosa: 1 - latente; 2- fulminante; 3- aguda; 4- crónica y 5- subaguda, una modalidad que correspondía al 70% de los casos.

El siglo veinte y la introducción de antibióticos

El término "antibiótico" fue inicialmente utilizado por Selman-Abraham Waksman (1888-1973) en 1942, adaptándolo a la sustancia que producen algunos microorganismos, que antagoniza el desarrollo de otros microorganismos. Al inicio de la década de 1940, la sulfanilamida, un compuesto sintético, empezó a utilizarse en los casos de endocarditis infecciosa y se produjeron algunos informes de éxito terapéutico, aunque en la mayoría de los casos la mejoría era transitoria. No fue hasta la disponibilidad universal de la penicilina, gracias a los esfuerzos pioneros del inglés y ganador del premio Nobel, Howard Florey (1898-1968) y de los americanos cómo Martin Henry Dawson (1896-1945), que la eficiencia en el tratamiento de la endocarditis infecciosa inició su asenso [8].

Sin embargo, el descenso en la mortalidad de aproximadamente un 30% (factor dependiente del uso de penicilina) trajo algunas preocupaciones en cuanto a la sobrevida de la infección y la disminución en la calidad de vida, debido al empeoramiento de la cardiopatia de base. Como

consecuencia, los dañinos efectos morfológicos de la endocarditis infecciosa aumentaron la investigación en los fundamentos de la intervención directa de aquellas lesiones valvulares severas y sintomáticas.

En 1960, se estableció que la endocarditis infecciosa era una enfermedad curable. Andrew G. Wallace *et al.* [9] de la Universidad de Duke, comprendió que la retirada de la válvula infectada y su sustitución por una válvula protésica, constituían un enfoque para la causa de la manifestación clínica. Esta innovación asistencial, marcó el inicio de la asociación entre la válvula protésica y la endocarditis infecciosa, ambas como terapéutica así como etiopatogénica (endocarditis protésica). Subsecuentemente, la indicación quirúrgica en casos con endocarditis infecciosa con insuficiencia cardiaca grado III/IV (la recomendación más frecuente) redujo la mortalidad al 10%.

Gracias a los estudios de Inge Edler (1911-2001) y Hellmuth Hertz (1920-1990), en 1950, y la subsecuente contribución de Harvey Feigenbaum, la ecocardiografía inició con el uso de la ultrasongrafía para evaluar la insuficiencia mitral después de la comisurotomia y el derrame pleural. La introducción de técnicas de imagen en el campo de la endocarditis infecciosa, representó una importante evolución en el apoyo de los criterios diagnósticos de la endocarditis infecciosa, al unir los criterios clínicos tradicionales y las imágenes y cálculos determinados por el avance tecnológico en la ecocardiografía.

En 1990, David Durack et al [10], de la Universidad de Duke, dio otra magnifica contribución en el conocimiento de la endocarditis infecciosa, a través de la sistematización diagnóstica, que elevó a la ecocardiografía como un método determinante de criterio diagnóstico mayor. Los criterios de la Universidad de Duke, utilizaron la estrategia de Thomas Duckett Jones (1899-1954) de subdividir los criterios en mayores y menores. Varios estudios validaron estos criterios; sin embargo se percibieron algunos fallos. En el año 2000, Jennifer S. Li, y colaboradores [11], presentaron un versión perfeccionada de los criterios de Duke de 1994, alcanzando un extraordinario avance en el campo de conocimiento de la endocarditis infecciosa del siglo veinte.

ANTECEDENTES GENERALES

El término endocarditis infecciosa (EI) denota una infección de la superficie del endocardio e implica la presencia física de microorganismos en la lesión [12]. Aunque en la mayoría de los casos, las más frecuentemente afectadas son las válvulas cardíacas, esta enfermedad también puede ocurrir en defectos del septo o en la pared de endocardio.

Se ha descrito [13] que el desarrollo de la EI probablemente requiera de la ocurrencia simultánea de varios factores independientes e influenciados por el huésped. La superficie valvular debe alterarse de manera que produzca un lugar adecuado para la adhesión y colonización bacteriana. Estás alteraciones resultan en el depósito de plaquetas, fibronectina y

fibrina, entre otros; posteriormente las bacterias deberán alcanzar éste punto y adherirse e invadir el tejido involucrado, para producir la colonización y persistencia. Después de la colonización, la superficie es recubierta rápidamente con una capa protectora de fibrina y plaquetas de tal manera que se produzca un ambiente propicio para la posterior multiplicación bacteriana y crecimiento de la vegetación [14, 15].

Han existido varias clasificaciones de la EI. Clásicamente, la división se basa en una progresión de la enfermedad sin tratamiento (aguda - subaguda- crónica) [16], pero desde la introducción del tratamiento antibiótico, se prefiere una clasificación basada en el agente etiológico y/o cuadro clínico (nativa-protésica-dispositivo) [17].

La EI es una enfermedad grave con una elevada morbilidad, parcialmente relacionada con la necesidad cirugía cardíaca mayor, secuelas neurológicas y estancias hospitalarias prolongadas con tratamiento intravenoso. Las tasas de mortalidad presentan un amplio rango que varía desde el 13% al 25% durante el ingreso hospitalario, con un aumento del 9% al 20% durante el primer año de seguimiento [18-21]. El conocimiento de la epidemiología de la EI es esencial, ya que diferentes organismos producen un gran variedad de complicaciones que requieren diferentes tratamientos y profilaxis.

EPIDEMIOLOGÍA

A pesar de la dificultad en su evaluación, la incidencia de la EI fue determinada en 12.7 casos por 100,000 habitantes en un estudio realizado en Estados Unidos entre 1998 y 2009 [19]. El programa de vigilancia cardíaca europea (EHS por sus siglas en inglés), iniciado por los hallazgos de la Sociedad Europea de Cardiología, muestra que la endocarditis activa, no es una enfermedad frecuente; los pacientes con endocarditis, representa únicamente el 3.2% del total de la población en el programa EHS con enfermedad valvular (EV). Estos hallazgos son congruentes con los resultados de un reciente estudio de vigilancia Francés, que estimó la incidencia en 31 casos por millón de adultos [22].

España no cuenta con estudios poblacionales, por lo que no es posible determinar la incidencia exacta de ésta enfermedad; pero estudios realizados en grandes centros hospitalarios [21] ha reportado una incidencia estimada de 6.4 casos por 100,000 habitantes/año, 0.8 casos por 1000 ingresos y 3.5% de todos los episodios de bacteremia significativos.

Dentro de los estudios hospitalarios, la epidemiología de la EI ha cambiado principalmente en tres aspectos durante las últimas cinco décadas [20]. Primero, los paciente son significativamente mayores (1980: edad media 45.3, IC 40.2- 50.5 vrs 2000: edad media 57.2, IC 54.7- 59.7, $p<0.001$).

Segundo, la mayoría de los casos son varones (1970: 58.6%, IC 54.3- 63.0 vrs 2000: 66.3%, IC 63.6- 69.0, p<0.01); y tercero, el porcentaje de casos de EI sobre válvula protésica ha aumentado (1960: 8.4%, IC -3.8- 20.5 vrs 2000: 22.9%, IC 19.1 - 26.7, p=0.05).

Debe de considerarse que, la EI asociada a la asistencia sanitaria, ha surgido de forma secundaria a la introducción de nuevas modalidades terapéuticas (catéteres intravenosos, marcapasos, derivaciones de diálisis, entre otros) y ha influenciado a la microbiología de la EI [23-25].

MICROBIOLOGÍA

Existen una gran variedad de microorganismos como agentes causales de a endocarditis infecciosa (EI); los estafilococos y estreptococos representan el origen de la mayoría de los casos [26]. Sin embargo, han ocurrido varios cambios importantes en la microbiología a lo largo del tiempo; la tasa EI por *Streptococcus viridans* ha disminuido considerablemente (1960:27.4%, IC 18.4-36.4 vrs 2000: 17.6%, IC 15.7-19.5, p<0.05); mientras que la frecuencia de EI por otros microorganismos como el *Staphylococcus aureus* (1960: 18.1% IC 9.4- 26.7 vrs 2000s: 29.7%, IC 26.2- 33.3, p<0.05); y los *Staphylococcus* coagulasa negativa (1960: 2.4%, IC 0.8-5.5 vrs 2000: 10.0%, IC 8.6-11.3, p<0.01) ha aumentado.

Datos más recientes muestra que la tasa de EI con hemocultivos negativos (1980s: 23.1%, CI 15.0- 31.3 vs 2000s: 14.2% CI 9.9- 18.2; p=0.01) y la endocarditis enterocócica (1980: 6.8%, CI 5.4- 8.2 vrs 2000: 10.5%, CI 8.9- 12.1, p<0.001) han aumentado en las últimas décadas [20].

Debido a que las especies de *Enterococcus* son una causa frecuente y en aumento de bacteremia en muchas instituciones [27], consideramos que su revisión merecen un apartado especial.

Endocarditis Enterocócica

El género Enterococcus incluye microorganismos que históricamente han sido relacionados con el género *Streptococcus*, y su documentación inicia está relacionada con la de los "estreptococos de origen fecal" o "enterococos". Después de la introducción de los métodos moleculares, los enterococos han pasado por cambios considerables en su taxonomía, que inició con su separación del género *Streptococcus* y el reconocimiento de los *Enterococcus* como un género diferente en 1984 [28].

El continuo uso de los métodos moleculares, ha permitido grandes avances en la clasificación de los enterococos, que ha dado como resultado, el reconocimiento de 49 especies de enterococos [16, 29]

Los enterococos son cocos Gram-positivos, anaerobios facultativos, que forman cadenas de varias longitudes; todos los enterococos son parte del grupo D de Lancefield; son catalasa negativos y no móviles. Crecen bien en

sodio azida, 40% de bilis, 6.5% de cloruro de sodio, y 0.1% de azul de metileno; hidrolizan la esculina en la presencia de sales biliares (test de bilis-esculina) y pueden sobrevivir a 56ºC durante 30 minutos o a un pH de 9.6 [28].

Después de su incubación durante 24 horas en agar sangre, las colonias son usualmente de 1 a 2 mm de diámetro. Los enterococos son anaerobios facultativos con un metabolismo hemofermentador, que resulta en la producción de ácido láctico [L-(+)] como su principal producto de fermentación [29].

Debido a su habilidad para fermentar una amplia gama de carbohidratos en ácido láctico, a los enterococos se les conoce como bacterias típicas acido lácticas (ALB). No producen gas. Estos microorganismos son usualmente capaces de crecer a temperaturas que varían de 10 a 45ºC con un crecimiento óptimo a 35 - 37ºC [30].

Varias características intrínsecas de los enterococos les permiten crecer y sobrevivir bajo circunstancias difíciles y sobrevivir casi en todas partes, colonizando varios nichos ecológicos [31]. Estos microorganismos tienen una amplia distribución en la naturaleza y pueden encontrarse en la tierra, vegetación, agua, comida y animales.

En los humanos, se encuentran principalmente en el tracto gastrointestinal y es menos frecuente encontrarlos en otros sitios como el

tracto genitourinario, la cavidad oral, la piel y especialmente en el área perineal [31]. La prevalencia de las diferentes especies de enterococos varía de acuerdo con el huésped y parece estar relacionado con la edad, dieta y otros factores que pueden estar relacionados con los cambios de las condiciones fisiológicas, como pueden serlo las enfermedades de base y tratamientos antibióticos previos.

Los enterococos están considerados como los cocos Gram-positivos más abundantes que colonizan el intestino, siendo el *E. faecalis* una de las especcies bacterianas más frecuentes de este sistema [29]. Debido a que los enterococos son patógenos oportunista, la incidencia de cada especie encontrada en las infecciones humanas probablemente refleja la distribución de las diferentes especies de *Enterococcus* en el tracto gastrointestinal.

Se cree que el tracto gastrointestinal representa un importante reservorio para las cepas asociadas con la enfermedad; esta localización les permite migrar y causar infecciones, así como su diseminación hacia otros huéspedes y el ambiente [16].

Por otra parte, el alto número de enterococos en la heces y su habilidad para resistir diferentes condiciones químicas y físicas, así como su habilidad para sobrevivir en el ambiente, implican que los enterococos

pueden ser utilizados como indicadores de contaminación fecal y como controles de calidad en la higiene de la comida, agua y lácteos [32].

La presencia de los enterococos como miembros de la microbiota intestinal humana y su relación con la presencia de enterococos en la comida y su seguridad para el consumo humano, han sido ampliamente revisados [33, 34].

Los enterococos son microorganismos comensales que actúan como agentes oportunistas, provocando una gran variedad de infecciones en humanos. El *Enterococcus faecalis* es el patógeno humano más frecuente, sin embargo, la prevalencia del *Enterococcus faecium* ha aumentado significativamente en las infecciones relacionadas con la asistencia sanitaria. Todas las demás especies de enterococo representan menos del 5% de las infecciones [35, 36]. Los sitios de infección enterocócica más frecuente son el tracto urinario, el torrente sanguíneo, el endocarido, quemaduras, infecciones de la herida quirúrgica, abdomen, tracto biliar y los catéteres así como otros dispositivos implantables [16, 29].

Durante décadas, han pasado de ser considerados como bacterias virtualmente inocuas a ser patógenos asociados a infecciones relacionadas con la asistencia sanitaria con importantes mecanismos de resistencia antibiótica, que contribuyen significativamente en la morbi y mortalidad de los pacientes, así como a un aumento en el coste sanitario [27].

Cambios en la dinámica entre la relación bacteria comensal y huésped, como los ocasionados por el uso de antibióticos de amplio espectro, daño en el huésped o disminución en la inmunidad del huésped, han podido permitir a éstas bacterias ganar acceso a sitios extra-intestinales del huésped y provocar infección. Por lo tanto, pacientes ancianos con enfermedades de base graves y otros pacientes severamente inmunodeprimidos con largas estancias hospitalarias, portadores de dispositivos invasivos y/o que han recibido tratamiento antibiótico de amplio espectro, son los que presentan mayor riesgo de adquirir una infección enterocócica [27, 37, 38].

Se han identificado varios factores de potencial virulencia, que pueden tener un papel en la patogénesis de las infecciones enterocócicas, dentro de las que se incluyen: adhesinas de superficie (proteínas de superficie enterocócica) y sustancias de agregación (SA); secreción de toxinas citolisina/hemolisina, secreción de proteasas gelatinas y proteasa serina, antígeno A del *Enterococcus faecalis* (EfaA), cápsula enterocócica, pared celular polisacárida y el superóxido extracelular [39-41]. Sin embargo, ninguna de las anteriores ha demostrado tener una contribución superior en la virulencia enterocócica en humanos.

La habilidad para formar biopelículas, recientemente ha sido considerada como la propiedad de virulencia más importante de éstos microorganismos, permitiendo la colonización de superficies biológicas inertes que al mismo tiempo les protege de la acción de sustancias antimicrobianas y de la adhesión e invasión de células del huésped (35). La formación de biopelículas puede ser de particular importancia en el desarrollo de endocarditis, al igual que en las infecciones relacionas con dispositivos médicos implantables [27, 42, 43].

En los hospitales de Estados Unidos, los enterococos son los segundos microorganismos responsables de las infecciones relacionadas con el catéter, del tracto urinario y de las infecciones de piel y partes blandas [44, 45]. Sin embargo, en otros tipos de infecciones como pueden ser la endocarditis y la bacteriemia, los enterococos pueden claramente causar una infección grave y potencialmente mortal. Además, el origen de hasta un tercio de las endocarditis infecciosas en España [46] están relacionadas con la asistencia sanitaria.

El porcentaje de pacientes con bacteriemia enterocócica que desarrollan EI oscila entre un 3% a un 10% [38, 47, 48]. Las diferencias en las cifras están parcialmente sesgados por la selección de estudios poblacionales y por los métodos utilizados para confirmar la endocarditis. Algunos autores analizan a todos los pacientes con bacteriemia enterocócica [27, 43, 49],

43), mientras que otros solo incluyen a los pacientes con al menos 2 hemocultivos positivos [23, 38, 42, 50].

El *E. faecalis* sigue siendo la causa más frecuente de endocarditis enterocócica. Esta afección de la válvula cardíaca, típicamente ocurre en pacientes ancianos [42, 51, 52]. El origen de la bacteriemia que provoca la endocarditis, usualmente se encuentra en el tracto genitourinario o gastrointestinal. La afección de las válvulas izquierdas es mucho más frecuente que la afección de las válvulas derechas. Se ha registrado un aumento significativo en la endocarditis sobre válvula protésica, lo cual esté probablemente relacionado con el aumento en el uso de éstas prótesis en adultos de edad avanzada, con un riesgo de desarrollar bacteriemia enterocócica significativamente mayor [42, 53]. Se ha descrito recientemente que entre un 15% a un 39% de los casos de endocarditis enterocócica, están relacionados con la asistencia sanitaria [51, 54]. El cuadro clínico de la endocarditis enterocócica, usualmete refleja un cuadro subagudo de infección, caracterizado por insuficiencia cardíaca, más que por eventos embolicos [51]; sin embargo, la enfermedad progresiva rápida, también puede ocurrir, por lo que el diagnóstico temprano de la EI enterocócica es esencial para mejorar el pronóstico y frecuentemente requiere de la realización de un ecocardiograma transesofágico (ETE) [17, 55]. Sin embargo, la utilización sistemática de ETE en todos los pacientes con bacteriemia enterocócica no está libre de complicaciones.

DIAGNÓSTICO

El diagnóstico de la endocarditis debe realizarse lo antes posible para iniciarse el tratamiento adecuado e identificar a los pacientes con alto riesgo de desarrollar complicaciones, que pueden beneficiarse de un manejo quirúrgico precoz.

La variabilidad en la presentación clínica de la EI requiere de una estrategia diagnóstica que sea tanto sensible para la detección de la enfermedad y específica para su exclusión. En 1994, Durack y colaboradores [10] de la Universidad de Duke, propusieron un esquema diagnóstico llamado "los criterios de Duke", el cual clasificaba a los pacientes con sospecha de EI en 3 categorías: caso "definitivo" identificado por hallazgos clínicos o de anatomía patológica (EI probada durante la cirugía o autopsia); casos "posibles" (que no cumplían los criterios de endocarditis definitiva); y casos "rechazados" (sin evidencia patológica de EI durante la autopsia o cirugía, con resolución rápida del síndrome clínico con o sin tratamiento, o la presencia de un diagnóstico alternativo).

El diagnóstico de la EI se basa en la presencia de criterios clínicos mayores o menores. Los criterios mayores de clasificación de Duke incluyen; EI documentada durante la cirugía o la autopsia (definición patológica) o bien

por criterios microbiológicos claramente definidos (bacteriemia o fungemia de alto grado) más datos ecocardiográficos (clínicamente definido)

Los criterios de Duke incorporaron los hallazgos ecocardiográficos en la estrategia diagnóstica. La definición de criterio mayor está dada únicamente a 3 hallazgos ecocardiográficos: la presencia de una masa móvil, ecodensa adherida a la válvula o a la pared del endocardio; la presencia de un absceso perianular; o la presencia de una nueva dehiscencia sobre una válvula protésica.

Otros seis hallazgos frecuentes, pero menos específicos también fueron incluidos como criterios menores al esquema original de Duke: bacteriemia o fungemia intermitente, fiebre, eventos embolicos mayores, fenómenos vasculares no embolicos, valvulopatía previa, uso de drogas por vía parenteral y anomalías ecocardiográficas que no cumplieran con los hallazgos de criterio mayor

Por lo tanto, la EI es una enfermedad grave que cuyo diagnóstico emplea una combinación de criterios clínicos, microbiológicos y de imagen [12, 16]. Sin embargo, la alta morbi y mortalidad, en parte son el resultado de una alta tasa (23-45%) de complicaciones embolicas a distancia [18, 21].

El fallo en la identificación de complicaciones metastásicas a distancia, puede llevar, erróneamente a la interrupción del tratamiento, desencadenando una recaída y pronóstico desfavorable. Los embolismos

infecciosos pueden ser asintomáticos y difíciles de reconocer [56], por lo que se recomienda el uso de múltiples técnicas de imagen [Tomografia Axial Computarizada (TAC), Resonancia Magnetica (RM) y ecografía] para su diagnóstico [57]. Sin embargo, éste abordaje diagnóstico conlleva a una considerable pérdida de tiempo, que involucra el transferir constantemente a un paciente severamente enfermo al departamento de radiología.

La 2-[18F]-fluoro-2-deoxi-D-glucosa (18F-FDG) tomografía por emisión de positrones (PET-TAC) es una técnica de imagen que nos permite identificar la captación de glucosa en áreas con un aumento en la actividad metabólica [58] y es ampliamente utilizada en los pacientes con enfermedades hemato-oncológicas. Debido a su alta sensibilidad, precisión anatómica y carencia de toxicidad [59, 60], su uso en el campo de las enfermedades infecciosas es prometedor. La posibilidad de rastrear todo el cuerpo en una sola prueba es de particular importancia para los médicos que se dedican a tratar pacientes con endocarditis infecciosa.

Los estudios que analizan el uso del PET-TAC en la evaluación de pacientes con endocarditis infecciosa son escasos, carecen de grupo control, evalúan a un pequeño número de pacientes o consisten en la descripción de un único caso [61, 62]. El campo de evaluación recientemente realizado por Saby *et al*, [63] está limitado al corazón y se detectaron una alta tasa de falsos negativos (33%).

APORTACIONES

Debido a la baja incidencia de la endocarditis infecciosa, los datos en cuanto a la presentación clínica, complicaciones y pronóstico han sido recogidos de series estudiadas durante largos periodos, realizados en un solo centro o durante periodos más cortos pero que involucran estudios multicéntricos y multinacionales. Consecuentemente los resultados, no necesariamente representan la situación actual de la endocarditis infecciosa en España, por lo que una de las principales aportaciones de la presente tesis doctoral es retratar la situación actual de la endocarditis infecciosa en España, así como presentar dos nuevas aportaciones en este campo: primero, el uso de un sistema de estratificación de riesgo de los pacientes con bacteriemia enterocócica que permite identificar a los pacientes con bajo riesgo de desarrollar endocarditis infecciosa; y segundo, el impacto clínico del uso sistemático de una técnica de imagen molecular (PET-TAC) para la detección de embolismos sépticos en pacientes con endocarditis infecciosa.

OBJETIVOS

I. Evaluar la situación actual de la Endocarditis Infecciosa en España.

1.1 Describir los cambios en la epidemiología y manifestaciones clínicas de la EI a nivel nacional.

1.2 Evaluar los factores de riesgo relacionados con la mortalidad a largo y corto plazo de la EI durante los primeros cinco años de nuestro estudio.

II. Evaluar las características epidemiológicas, microbiológicas y clínicas de la endocarditis enterocócica.

2.1 Identificar las características que puedan ayudar a identificar la EI entre los pacientes con bacteriemia por *Enterococcus*.

2.2 Comparar el pronóstico de la bacteriemia por enterococo en pacientes con y sin endocarditis, para poder evaluar las potenciales consecuencias de su diagnóstico erróneo.

2.3 Validar un modelo predictivo de pacientes con bacteriemia por enterococo con bajo riesgo de desarrollar EI.

III. Evaluar la realización sistemática de nuevas técnicas de imagen en pacientes con EI.

3.1 Evaluar el impacto clínico del uso sistemático del 18F-FDG PET-CT para el diagnóstico de embolismos sépticos en pacientes con EI. .

MATERIAL, MÉTODOS Y RESULTADOS

I. Epidemiología y pronóstico de la endocarditis infecciosa en España.

Desde enero 2008, varios grupos multidisciplinarios de 25 centros hospitalarios, han recogido de forma prospectiva todos los casos de EI diagnosticados de acuerdo con los criterios de Duke. En total, se reclutaron 1804 pacientes durante el periodo 2008-2012. La edad media de los casos fue de 69 años (IC, 55-77), el 68.0% eran hombres y el 37.1% de los casos eran nosocomiales o relacionados con la asistencia sanitaria. Los microorganismos Gram-positivos representaron el 78.8% de los casos, seguidos por los Gram-negativos (5.2%), hongos (2.4%), anaerobios (1.2%), infecciones polimicrobianas (1.9%) y de etiología desconocida (9.1%). Se realizó cirugía cardíaca en el 44.2% de los casos y la mortalidad durante el ingreso fue del 28.8%. En total, se completó el seguimiento al año en 1035 (80.6%) de los pacientes que sobrevivieron, de los cuales 69 necesitaron cirugía cardíaca (6.6%), 32 (3.1%) tuvieron una recurrencia y 116 (11.2%; relacionadas con la EI 31-8%) fallecieron.

Los factores de riesgo para la mortalidad durante el ingreso hospitalario fueron, la edad, antecedente de cirugía cardíaca, enfermedad cerebrovascular, fibrilación auricular, etiología por *Staphylococcus* o *Candida*, complicaciones intra cardíacas, insuficiencia cardíaca y shock séptico. Los factores de riesgo independientes, para la mortalidad durante

el año de seguimiento que se identificaron fueron: edad avanzada (OR, 1.02), neoplasia (OR, 2.46), insuficiencia renal (OR, 1.59), e insuficiencia cardíaca (OR, 4.42). La única intervención que ha demostrado tener un mayor impacto en la mortalidad al año de seguimiento ha sido la el tratamiento quirúrgico (OR, 0.44).

II. Factores de riesgo y pronóstico de la endocarditis infecciosa por *Enterococcus* spp.

Durante Septiembre 2003 y Octubre 2012, se realizaron dos estudios, uno de cohorte, prospectivo (incluyendo a todos los pacientes con BE) y otro de casos y controles (pacientes con y sin EI por *Enterococcus* spp.) en un centro hospitalario de nivel terciario.

Durante el período de estudio, se detectaron 1515 pacientes con BE y 65 casos de EI por *Enterococcus* spp. Los casos de endocarditis infecciosa por *Enterococcus* spp, representan el 4.29% de todos los episodios de BE, el 16.7% de todos los pacientes con BE a los que se les realizó una ecocardiografía transtorácica, y el 35.5% de todos los pacientes con BE a los que se les realizó una ecocardiografía transesofágica.

Posteriormente realizamos un índice predictivo de 12 puntos– NOVA score (por sus siglas en inglés)- para EI por *Enterococcus* spp, cuyas variables pudieran ser evaluadas a pie de cama del paciente; dichas variables incluyen el **N**úmero de hemocultivos positivos (3/3 hemocultivos positivos o la mayoría si se recogían más de 3), 5 puntos: **O**rigen desconocido de la bacteriemia, 4 puntos; Enfermedad **V**alvular previa, 2 puntos; y **A**uscultación de un soplo cardíaco, 1 punto (COR = 0.83). El mejor punto de corte, corresponde a una puntuación de ≥4 (sensibilidad, 100%; especificidad, 29%).

Una puntuación <4 puntos, sugiere que el riesgo para EI por *Enterococcus* spp. es muy bajo, y por lo tanto podría evitarse la realización de una ecocardiografía transesofágica. Dependiendo de la prevalencia local de la endocarditis infecciosa, la aplicación del índice NOVA, podría de forma confiable, evitar la realización de una ecocardiografía en el 14-27% de los pacientes con bacteriemia por *Enterococcus* spp.

III. Impacto clínico del uso del 18-FDG PET-CT en el diagnóstico de embolismos sépticos en pacientes con endocarditis infecciosa.

Estudio de cohorte prospectivo con 47 casos (EI definitivas a los que se les realizó PET-TAC) y 94 controles (EI definitivas a los que no se les realizo PET-TAC) durante Enero 2012 a Julio 2013 en un hospital terciario. Los resultados del PET-TAC fueron comparados con los hallazgos de las pruebas de imagen convencional y el seguimiento clínico. El PET-TAC mostró al menos 1 lesión en 35 pacientes (74.5%): en 18 pacientes se encontró una complicación embolica, en 8 había una captación en las válvulas o dispositivos cardíacos, un paciente tenia ambos hallazgos, 5 mostraron hallazgos incidentales no infecciosos y 3 fueron considerados como falsos positivos.

Los valores de validación para la eficacia del PET-TAC en el diagnóstico de lesiones sépticas a distancia fueron: sensibilidad, 100%, especificidad, 80%; valor predictivo positivo, 90%; y valor predictivo negativo, 100%. El PET-TAC fue la única técnica de imagen en mostrar un hallazgo positivo en 15 casos verdaderos positivos (55.5%). El uso sistemático del PET-TAC estuvo asociado a una reducción de 2 veces, en el número de recurrencias (9.6% vrs 4.2% p=0.25) y aumentó de manera significativa, el diagnóstico de complicaciones infecciosas (18% vrs 57.4%; p = 0.0001).

CONCLUSIONES

I. La endocarditis infecciosa es una enfermedad infrecuente pero grave, que comúnmente se presenta en pacientes de edad avanzada, con múltiples enfermedades de base y frecuentemente está relacionada con la asistencia sanitaria.

II. Los grupos multidisciplinarios, son esenciales para la optimización del manejo, tratamiento y pronóstico de esta grave enfermedad; la única intervención que ha demostrado tener un impacto en la mortalidad al año de seguimiento, es la intervención quirúrgica.

III. La prevalencia de la endocarditis infecciosa por Enterococcus, depende de si se considera a todos los casos de BE (4.3%), solo a los pacientes a los que se les realiza una ecocardiografía (16.7%), o si se considera únicamente a los pacientes a los que se les realiza una ecocardiografía transesofágica (35.5%).

IV. Dependiendo de la prevalencia local de endocarditis, la aplicación del índice de predicción NOVA a pie de cama del paciente, podría de forma confiable, evitar un 14-27% de las ecocardiografías en pacientes con bacteriemia por Enterococcus.

V. El PET-TAC es una forma efectiva de realizar el estudio de extensión en pacientes con EI, utilizando una sola técnica de imagen. Es fácil de realizar (< 2 horas), cómodo para el paciente y proporciona al clínico información del cuerpo completo.

VI.

El PET-TAC, permite el diagnóstico de significativamente más complicaciones infecciosas (18.0% vrs. 57.4%; p = 0.0001) y su utilización sistemática, muestra una tendencia hacia la reducción en el número de recurrencias (9.6 % vs. 4.2% p = 0.25) en pacientes con EI.

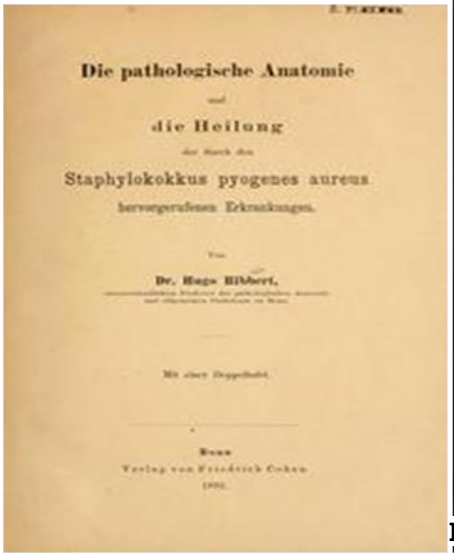
INTRODUCTION

Infective Endocarditis History Review

The very term "endocarditis", referring to an individual tissue and an inflammatory process, goes back to early-nineteenth-century with clinicians such as Broussais and Bouillaud, before the germ theory and the birth of bacteriology [1]. However, it was very difficult during that period to define endocarditis in a simple, unequivocal manner. There was not always a clear relationship between the ideas which late historians have supposed fed each other; the arguments were confusing, tortuous, circular, and dead-end. In his discussion of syphilis, Ludwik Fleck correctly pointed out that disease is a constructed, ever-changing phenomenon which constantly integrates new information and concepts [2].Thus, the term "endocarditis" continued to be used for a disease which underwent endless development throughout the nineteenth century. There was also modification of the aetiological links between anatomical abnormalities, clinical symptoms and observations during autopsy. The pathology of the disease was regularly reformulated and its definition varied from period to period and from country to country. The germ theory and the use of the microscope changed the view and concept of the disease at the end of the century. The fact of being able to "see" minute granulations hitherto invisible is not enough immediately to transform these granulations into an analytical tool.

The perception of the disease as a relatively coherent whole with aetiological symptoms, led, nevertheless, to laboratory experiments on diseases [3].

Hugo Ribbert (1855-1920) performed experiences of infective endocarditis induction. He injected *Staphylococcus aureus* cultured in potatoes into rabbits and identified bacterial colonies over particles on the surface of heart valves, especially in the *chordae tendineae* of the mitral valve. In the same year, WK Wyssokowitsch (1854-1912) obtained the colonization of bacteria injected into the bloodstream of rabbits after previous scarification of the aortic valve via the carotid artery.

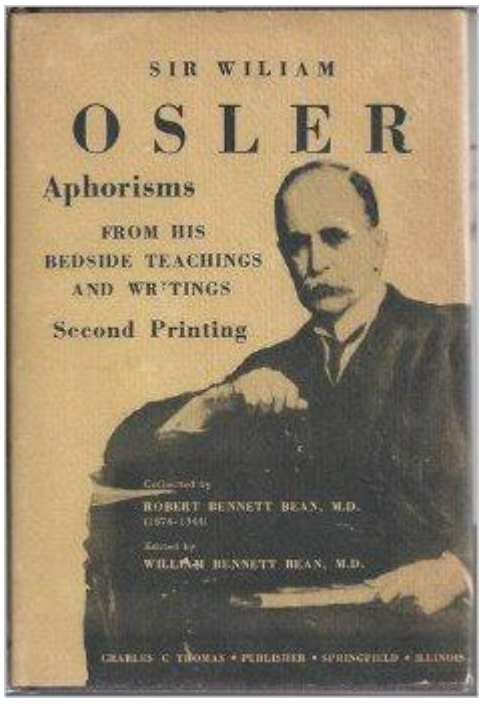

Die pathologische Anatomie
und
die Heilung
der durch den
Staphylokokkus pyogenes aureus
hervorgerufenen Erkrankungen.
Von
Dr. Hugo Ribbert,
Bonn
Verlag von Friedrich Cohen
1890.


Hugo Ribert (1855-1920)

Based on the collection of experiments in animals of the time, two conclusions were drawn: a) the anteriority of a nonbacterial thrombotic endocardiopathy; b) the colonization of this substrate by circulating bacteria. The 19th century ends associating valvular lesion, point of entry and circulation of microorganisms, fever and extra-cardiac manifestations under the synthetic diagnosis of infective endocarditis [1, 4].

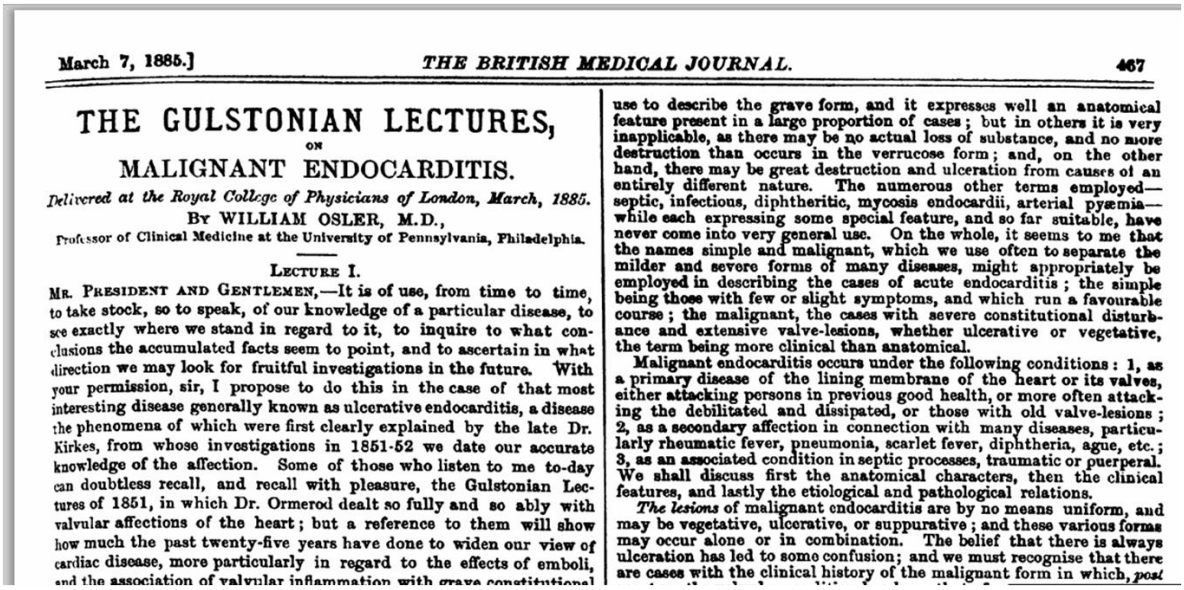
At the beginning of the twentieth century, before the dawning of the first rays of therapeutic hope, a few clinicians tried to shed light on the somewhat confused knowledge of endocarditis and to surmount the formidable problem of describing and classifying this complex disease [5].

William Bart Osler (1849-1919) is the eponym linked to infective endocarditis in general (Osler's disease) and one of its peripheral



manifestations (Osler's nodes).Osler perceived that there were simpler cases and more complex ones, and therefore, used discriminative terms such as ulcerative, malignant, septic and pyemic. He established that blood elements such as fibrin and platelets deposited on the damaged endocardium - substrate of nonbacterial thrombotic endocardiopathy - and constituted the nucleus of vegetation, devaluing the concept that it depended on secretions from the endocardium. Osler called attention to the diversity of microorganisms involved in the vegetation and collected evidence in favor of the primary characteristic of the presence of germs in the etiopathogeny of infective endocarditis at a time when the detection of living germs in blood cultures was incipient [6].

Stimulated by Osler's presentations, Lord Thomas Jeeves Horder (1871-1955)[5], a physician of the sovereigns of England, emphasized the pre-existence of valvulopathy and congenital cardiopathy, the importance of the oral and intestinal points of entry, the occurrence of mycotic aneurysm, the presence of splenomegaly and the identification of streptococcal etiology in more than 60% of the cases confirmed through necropsies [7].



March 7, 1885.] THE BRITISH MEDICAL JOURNAL. 467

THE GULSTONIAN LECTURES,
ON
MALIGNANT ENDOCARDITIS.

Delivered at the Royal College of Physicians of London, March, 1885.

By WILLIAM OSLER, M.D.,
Professor of Clinical Medicine at the University of Pennsylvania, Philadelphia.

LECTURE I.

MR. PRESIDENT AND GENTLEMEN,—It is of use, from time to time, to take stock, so to speak, of our knowledge of a particular disease, to see exactly where we stand in regard to it, to inquire to what conclusions the accumulated facts seem to point, and to ascertain in what direction we may look for fruitful investigations in the future. With your permission, sir, I propose to do this in the case of that most interesting disease generally known as ulcerative endocarditis, a disease the phenomena of which were first clearly explained by the late Dr. Kirkes, from whose investigations in 1851-52 we date our accurate knowledge of the affection. Some of those who listen to me to-day can doubtless recall, and recall with pleasure, the Gulstonian Lectures of 1851, in which Dr. Ormerod dealt so fully and so ably with valvular affections of the heart; but a reference to them will show how much the past twenty-five years have done to widen our view of cardiac disease, more particularly in regard to the effects of emboli, and the association of valvular inflammation with grave constitutional use to describe the grave form, and it expresses well an anatomical feature present in a large proportion of cases; but in others it is very inapplicable, as there may be no actual loss of substance, and no more destruction than occurs in the verrucose form; and, on the other hand, there may be great destruction and ulceration from causes of an entirely different nature. The numerous other terms employed—septic, infectious, diphtheritic, mycosis endocardii, arterial pyæmia—while each expressing some special feature, and so far suitable, have never come into very general use. On the whole, it seems to me that the names simple and malignant, which we use often to separate the milder and severe forms of many diseases, might appropriately be employed in describing the cases of acute endocarditis; the simple being those with few or slight symptoms, and which run a favourable course; the malignant, the cases with severe constitutional disturbance and extensive valve-lesions, whether ulcerative or vegetative, the term being more clinical than anatomical.

Malignant endocarditis occurs under the following conditions: 1, as a primary disease of the lining membrane of the heart or its valves, either attacking persons in previous good health, or more often attacking the debilitated and dissipated, or those with old valve-lesions; 2, as a secondary affection in connection with many diseases, particularly rheumatic fever, pneumonia, scarlet fever, diphtheria, ague, etc.; 3, as an associated condition in septic processes, traumatic or puerperal. We shall discuss first the anatomical characters, then the clinical features, and lastly the etiological and pathological relations.

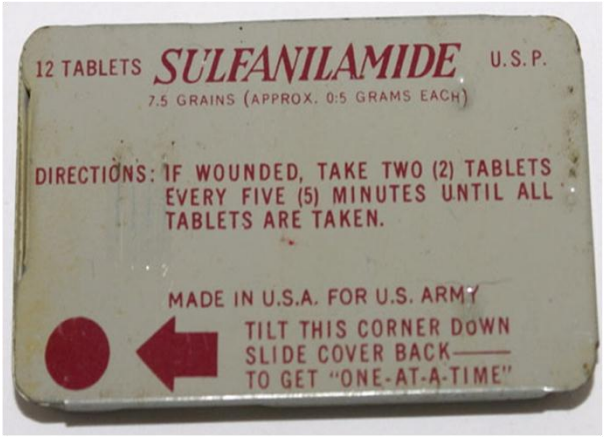
The lesions of malignant endocarditis are by no means uniform, and may be vegetative, ulcerative, or suppurative; and these various forms may occur alone or in combination. The belief that there is always ulceration has led to some confusion; and we must recognise that there are cases with the clinical history of the malignant form in which, *post*

Horder recognized five types of infective endocarditis: 1 - latent; 2- fulminant; 3- acute; 4- chronic and 5- subacute, a modality that corresponded to 70% of the cases.

The 20th Century and the introduction of antibiotics

The term "antibiotic" was initially employed by Selman-Abraham Waksman (1888-1973) in 1942, adapting it to a substance produced by microorganisms that antagonizes the development of other microorganisms.

In the beginning of the 1940s, sulfanilamide, a synthetic compound, started to be used in infective endocarditis and determined some reports of therapeutic success, although most cases showed a transient benefit, but



it was no after penicillin availability became universal, thanks to the pioneer efforts of Englishmen such as Nobel-prize winner Howard Florey (1898-1968) and Americans such as Martin Henry Dawson (1896-1945), that the treatment of infective endocarditis started an efficiency ascension [8]. However, the decrease in mortality to approximately 30% (penicillin-dependent fact) brought worries concerning surviving the infection and quality of life impairment due to the worsening in the previous cardiopathy. As a consequence, the deleterious morphological effects of

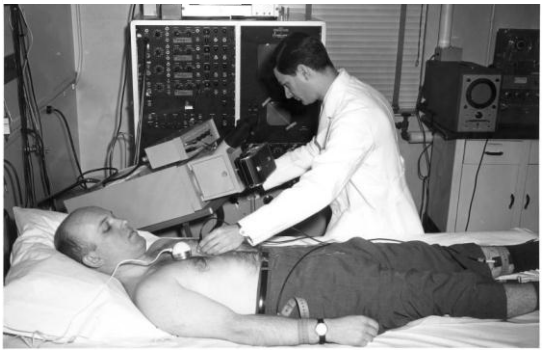
infective endocarditis increased the research on the foundations of the direct intervention on the severe and symptomatic valvular lesion.

In the 1960s, it was established that infective endocarditis was a curable disease. Andrew G. Wallace *et al.* [9] at Duke University, understood that the removal of the infected valve and its substitution by a valve prosthesis constituted an approach on the clinical manifestation cause.

This assistential innovation marked the beginning of the association of valvular prosthesis and infective endocarditis, both as therapeutics and etiopathogeny (endocarditis in prosthesis). Subsequently, the routine surgical indication in cases of infective endocarditis in cases with CHF grade III/IV (the most common recommendation) reduced mortality from 90% to 10%.

Thanks to the studies by Inge Edler (1911-2001) and Hellmuth Hertz (1920-1990), in the 1950s and the subsequent contribution of Harvey Feigenbaum, the echocardiography started with the use of ultrasonography to evaluate mitral failure after commissurotomy and pericardial effusion. Introduction of imaging techniques into the infective endocarditis field, represented a remarkable evolution in the support of the diagnostic rationale of infective

Dr. Harvey Feigenbaum



endocarditis by joining the traditional clinical data and images and calculations determined by technological advancement in echocardiography.

In the 1990s, David Durack et al. [10], from Duke University gave another magnificent contribution to the knowledge of infective endocarditis, through a diagnostic systematization that elevated echocardiography as a determinant method of a major criterion.

The Duke University criteria used the strategy utilized by Thomas Duckett Jones (1899-1954) of subdividing the criteria in major and minor ones. Several studies validated the new criteria; however, some gaps were perceived. In 2000, Jennifer S. Li, a professor of Pediatrics from Duke University and colleagues [11] presented a perfected version of the 1994 criteria from Duke University; completing the outstanding advancement in the frontier of knowledge on infective endocarditis in the 20th century.

GENERAL BACKGROUND

The term infective endocarditis (IE) denotes infection of the endocardial surface of the heart and implies the physical presence of microorganisms in the lesion [12]. Although the heart valves are affected most commonly, the disease also may occur within septal defects or on the mural endocardium.

It has been reported [13] that development of IE probably requires the simultaneous occurrence of several independent events and influenced by the host. The valve surface must be altered to produce a suitable site for bacterial attachment and colonization. These alterations result in the deposition of platelets, fibronectin, and fibrin, among others; then bacteria must reach this site and adhere to and invade the involved tissue to produce colonization and persistence. After colonization, the surface is covered rapidly with a protective sheath of fibrin and platelets to produce an environment conducive to further bacterial multiplication and vegetative growth [14, 15].

There have been several classifications for IE. Classically, the distinction was based on progression of the untreated diseases (acute – subacute – chronic)[16], but since the introduction of antibiotic treatment a classification based on the etiologic agent and/or clinical setting (native – prosthetic - device) is preferable [17].

IE is a severe disease with very significant morbidity, which is partially, related to the need for major heart surgery, neurologic sequelae, and prolonged hospital stay with intravenous therapy. Data on mortality vary widely, ranging from 13% to 25% during admission, with increases of 9% to 20% within one year after discharge [18-22]. Proper understanding of IE epidemiology is essential, as different organisms produce varied complications and may require different treatment and prophylaxis.

EPIDEMIOLOGY

Although difficult to assess, its incidence was shown to be 12.7 cases per 100,000 habitants in a study performed in the United States between 1998 and 2009 [19]. The Euro Heart Survey (EHS) program, initiated by the European Society of Cardiologyfindings, show that active endocarditis is not a common disease: patients with endocarditis account for only 3.2% of the total population in the EHS on valvular heart disease (VHD). These findings are consistent with the incidence of IE, which was estimated at only 31 cases per million adults in a recent French survey [23]. They are no population studies on EI in Spain, so it is not possible to determine the exact incidence of this disease in our country; but large tertiary care institution studies [21] have reported an estimated incidence of 6.4 cases per 100,000 inhabitants per year, 0.8 cases per 1,000 admissions and 3.5% of all episodes of significant bacteremia.

Among hospital-based studies, IE epidemiology changed mainly in three aspects over the last 5 decades [20]. First, patients are significantly older (1980s: mean age 45.3, CI 40.2- 50.5 vs 2000s: mean age 57.2, CI 54.7-59.7, $p<0.001$). Second, more are men (1970s: 58.6%, CI 54.3- 63.0 vs 2000s: 66.3%, CI 63.6- 69.0, $p<0.01$); and third, the percentage of IE cases occurring on prosthetic valves increased over time (1960s: 8.4%, CI -3.8-20.5 vs 2000s: 22.9%, CI 19.1 - 26.7, $p=0.05$).

It has to be notice that, the health care-associated IE, has emerged secondary to the introduction of new therapeutic modalities (intravenous catheters, pacemakers, dialysis shunts, among others) and has also influenced the microbiology of IE [24-26].

MICROBIOLOGY

A great variety of microorganisms can cause infective endocarditis (IE); staphylococci and streptococci account for the majority of cases [27]. However, there have been significant changes in microbiology over time; *Streptococcus viridans* IE markedly decreased in percentage over time (1960s:27.4%, CI 18.4-36.4 vs 2000s: 17.6%, CI 15.7-19.5, $p<0.05$); meanwhile, frequency distribution of other microorganism like *Staphylococcus aureus* IE (1960s: 18.1% CI 9.4- 26.7 vs 2000s: 29.7%, CI 26.2- 33.3, $p<0.05$);and coagulase-negative *Staphylococcus* IE (1960s: 2.4%, CI 0.8-5.5 vs 2000s: 10.0%, CI 8.6-11.3, $p<0.01$) have increased.

More recently, culture negative IE decreased (1980s: 23.1%, CI 15.0- 31.3 vs 2000s: 14.2% CI 9.9- 18.2; p=0.01) while enterococcal IE percentage increased significantly over the last decade (1980s: 6.8%, CI 5.4- 8.2 vs 2000s: 10.5%, CI 8.9- 12.1, p<0.001). Because *Enterococcus* species is an increasingly common cause of bloodstream infections (E-BSI) in many institutions we considered it should be separately analyzed [28].

Enterococcal IE

The genus Enterococcus includes microorganisms that have a historical connection with the genus Streptococcus, and their initial documentation is related to the “streptococci of fecal origin” or “enterococci". After the introduction of molecular methods, the enterococci have undergone considerable changes in taxonomy, which started with the splitting of the genus Streptococcus and the recognition of Enterococcus as a separate genus in 1984. The continuous use of molecular approaches has allowed major developments in the classification of the enterococci, resulting in the recognition of 49 enterococcal species [16, 29].

The enterococci are Gram-positive, facultatively anaerobic oval cocci that form chains of various lengths; all enterococci are in the Lancefield group D; they are catalase-negative and non-motile. They grow well in sodium azide (SF broth), 40% bile, 6.5% sodium chloride, and 0.1% methylene

blue, they hydrolyze esculin in the presence of bile salts (bile-esculin [BE] test)and can survive at 56°C for 30 minutes or at a pH of 9.6 [30].

After growth on blood agar medium for 24 h, colonies are usually between 1 and 2 mm in diameter. Enterococci are facultative anaerobes with a homofermentative metabolism that results in the production of L-(+)-lactic acid as the major end product of glucose fermentation [29]. Because of their ability to ferment a wide range of carbohydrates to lactic acid, the enterococci are referred to as typical lactic acid bacteria (LAB). Gas is not produced. These microorganisms are usually able to grow at temperatures ranging from 10 to 45°C with optimum growth at 35 to 37°C [31].

Several intrinsic characteristics of the enterococci allow them to grow and survive under harsh conditions and persist almost everywhere, colonizing several ecological niches [32]. These microorganisms are widespread in nature and can be found in soil, plants, water, food, and animals. In humans, they are predominantly inhabitants of the gastrointestinal tract and are less commonly found in other sites, such as the genitourinary tract, the oral cavity, and skin, especially in the perineal area [32]. The prevalence of the different enterococcal species appears to vary according to the host and is also influenced by age, diet, and other factors that may be related to changes in physiologic conditions, such as underlying diseases and prior antimicrobial therapy. Enterococci are considered among the most abundant Gram-positive cocci colonizing the intestine,

with *E. faecalis* being one of the most common bacterial species recovered from this site [33]. Since the enterococci are opportunistic pathogens, the incidence of each species found in human infections probably reflects the distribution of the different species of *Enterococcus* in the human gastrointestinal tract. This site is believed to represent an important reservoir for strains associated with disease; from this location, they may migrate to cause infections and can also disseminate to other hosts and to the environment [16].

On the other hand, the occurrence of high numbers of enterococci in the feces and their ability to resist different chemical and physical conditions and to survive in the environment imply that the enterococci can be used as indicators of fecal contamination and of the hygienic quality of food, milk, and drinking water [34]. The occurrence of enterococci as members of the intestinal microbiota of humans and the relationship between the presence of enterococci in foods and human safety have been extensively reviewed [35-38].

The enterococci are commensal microorganisms that act as opportunistic agents causing a variety of infections in humans. *Enterococcus faecalis* is the most common human pathogen, but *Enterococcus faecium* has become increasingly prevalent in hospital-acquired infections. All the other enterococcal species together constitute less than 5% of enterococcal infections [39, 40]. These other species associated with human infections

include *Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus avium, Enterococcus cecorum, Enterococcus durans, Enterococcus hirae, Enterococcus malodoratus, Enterococcus mundtii, Enterococcus pseudoavium and Enterococcus raffinosus.*

The enterococci most commonly infect the urinary tract, bloodstream, endocardium, burn and surgical site wounds, abdomen, biliary tract, and catheters and other implanted medical devices [16, 33].

Over the last decades, they have emerged from being considered virtually harmless bacteria to medically important multiple-antibiotic-resistant health care-associated pathogens that contribute significantly to patient morbidity and mortality as well as health care costs [28]. Changes in the dynamics of the commensal host-bacterial relationship, such as those promoted by the use of broad-spectrum antibiotics, host injury, or diminished host immunity, could allow these bacteria to gain access to extraintestinal host sites and cause infection. Therefore, elderly patients with serious underlying diseases and other severely ill immunocompromised patients who have been hospitalized for prolonged periods, have been treated with invasive devices, and/or have received broad-spectrum antimicrobial therapy are at higher risk to acquire enterococcal infections [28, 41, 42].

Several potential virulence factors that may play a role in the pathogenesis of enterococcal infections have been identified in enterococcal isolates, including the surface adhesinsEsp (Enterococcal surface protein) and aggregation substance (AS), secreted toxin cytolysin/hemolysin, secreted proteases gelatinase and serine protease, MSCRAMM Ace (Adhesin to collagen of E. faecalis), E. faecalis antigen A (EfaA), enterococcal capsule, cell wall polysaccharides, and extracellular superoxide [43-45]. Nevertheless, none has been established as making a major contribution to enterococcal virulence in humans.

The ability to form biofilms has recently been listed among the most prominent virulence properties of these microorganisms, allowing colonization of inert and biological surfaces while protecting against antimicrobial substances and mediating adhesion and invasion of host cells [46]. Biofilm formation may be of particular importance in the development of endocarditis, as well as implant and other medical-deviceassociated infections [28, 47, 48].

In hospitals in the United States, enterococci are the second most common organisms recovered from catheter-associated infections of the bloodstream and urinary tract, and from skin and soft-tissue infections [49, 50].However, for other types of infections, most notably endocarditis and bacteremia, enterococci can clearly cause serious and often life-threatening disease. In addition, up to one-third of infective endocarditis

patients in Spain [51] become infected through contact with the health care settings.

The percentage of patients with E-BSI who have infective endocarditis (IE) is estimated to be between 3% and 10% [42, 52, 53]. The differences in these values are at least partially biased by the study population selected and by the methods used to confirm endocarditis. Some authors analyzed all patients with E-BSI [28, 48, 54], whereas others only included patients with at least 2 positive blood cultures [24, 42, 55, 56].

E. faecalis remains the more common cause of enterococcal endocarditis than *E. faecium*. These heart valve infections typically occur in older patients [55, 57, 58]. The initial source of bacteremia leading to endocarditis is usually the genitourinary or gastrointestinal (GI) tract. Left-sided involvement is much more common than right-sided involvement. Prosthetic valve enterococcal endocarditis has been increasingly noted, which is perhaps related to the increasing use of these prostheses in older adults who are at an inherently higher risk for enterococcal bacteremia [55, 59]. In one retrospective analysis of a large endocarditis database [55], an equal number of women and men had enterococcal endocarditis, although enterococcal endocarditis is typically reported more often in men than women [57]. A recent large-case series of enterococcal endocarditis reported that between 15% and 39% are healthcare-associated [55, 57].

The clinical picture of enterococcal endocarditis is usually one of subacute infection characterized by heart failure, rather than embolic events [57]; however, rapidly progressive disease can also occur.

Establishing an early diagnosis of enterococcal IE it is essential to improve the outcome of these patients and most frequently requires transesophagealechocardiography (TEE) [17, 60]. However, using TEE in all patients with E-BSI is far from easy, costly, time-consuming, and subject to complications.

DIAGNOSIS

The diagnosis of endocarditis must be made as soon as possible to initiate therapy and identify patients at high risk for complications who may be best managed by early surgery.

The variability in clinical presentation of IE requires a diagnostic strategy that is both sensitive for disease detection and specific for its exclusion across all forms of the disease. In 1994, Durack and colleagues, [10] from Duke University Medical Center proposed a diagnostic schema termed the Duke criteria, which stratified patients with suspected IE into 3 categories: “definite” cases, identified either clinically or pathologically (IE proved at surgery or autopsy); “possible” cases (not meeting the criteria for definite IE); and “rejected” cases (no pathological evidence of IE at autopsy or

surgery, rapid resolution of the clinical syndrome with either no treatment or short-term antibiotic therapy, or a firm alternative diagnosis).

A diagnosis of IE is based on the presence of either major or minor clinical criteria. Major criteria in the Duke strategy included IE documented by data obtained at the time of open heart surgery or autopsy (pathologically definite) or by well-defined microbiological criteria (high-grade bacteremia or fungemia) plus echocardiographic data (clinically definite).

The Duke criteria incorporated echocardiographic findings in the diagnostic strategy. Major diagnostic weight was given to only 3 typical echocardiographic findings: mobile, echodense masses attached to valvular leaflets or mural endocardium; periannular abscesses; or new dehiscence of a valvular prosthesis.

Six common but less specific findings of IE also were included as minor criteria in the original Duke schema: intermittent bacteremia or fungemia, fever, major embolic events, non-embolic vascular phenomena, underlying valvular disease or injection drug use, and echocardiographic abnormalities that did not full fill the typical diagnosis of valvular vegetations, abscesses, or dehiscence.

Therefore, IE is a severe disease that is diagnosed using a combination of clinical, microbiological, and imaging criteria [10, 12].However, morbidity and mortality are significant, in part as a result of the high rate of distant embolic complications (23-45%) [18, 21].

Failure to identify metastatic complications may lead to early interruption of therapy, thus triggering relapse and unfavorable outcome. Infectious embolisms can be asymptomatic and difficult to recognize [61], with the result that systematic performance of multiple imaging techniques (computed tomography [CT], magnetic resonance imaging [MRI], and ultrasonography) has been recommended for all patients with IE[62]. However, this approach is time-consuming and cumbersome and involves frequent transfer of a very ill patient to the radiology department.

2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) positron emission tomography (PET)–CT is widely used in patients with onco-hematological conditions, since it can identify glucose uptake in areas with increased metabolic rate [63]. It has a promising role in infectious diseases, owing to its high sensitivity, anatomical precision, and lack of toxicity [64, 65]. The possibility of scanning the whole body with a single test is particularly appealing for clinicians treating patients with IE.

Studies analyzing PET-CT for the evaluation of patients with IE are scarce, lack a control group, evaluate a small number of patients, or consist of

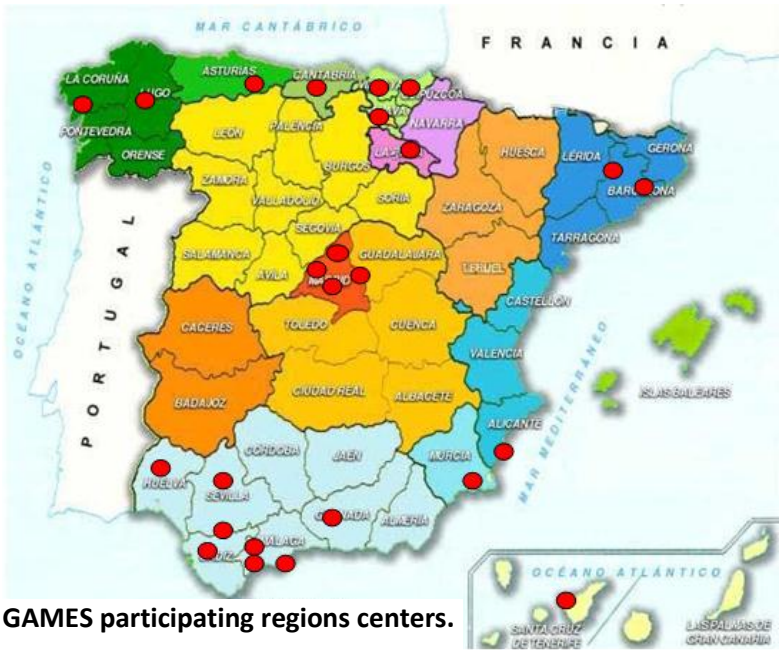
case reports [66, 67]. The field of examination in the recent report by Saby et al[68] was limited to the heart, and a high incidence of false-negative results were detected (33%).

Shortage in the literature

Overall, due to the low incidence of IE, data on clinical presentation, complications, and outcome are from series collected over prolonged periods, in single centers, or over shorter periods in multicenter, multinational studies. Consequently, they do not necessarily represent the current situation in Spain. Hence arising the need to create an infective endocarditis study group.

GAMES STUDY GROUP

The Spanish collaboration on endocarditis (GAMES) consists of multidisciplinary groups dedicated to improving the management of IE. These hospital-based endocarditis groups usually include microbiologists, infectious disease physicians, echocardiographers, heart surgeons, and cardiologists. Centers with no cardiac surgery unit had to be able to follow patients referred elsewhere for surgery. These groups prospectively recorded all consecutive episodes of IE at their institutions and collected the data according to a pre-established clinical form with common standard definitions [10, 16, 17]. At discharge, the clinical forms were sent to the coordinating center or data were entered directly by the investigators through a secure data entry system. In the coordinating center, specialized clinicians and data managers reviewed the data for accuracy and contacted the referring centers, if necessary, for queries and clarifications. Enrollment began in January 2008.



GAMES participating regions centers.

DEFINITIONS

IE was defined according to the modified Duke criteria [10]. Episodes were classified into four distinct categories representing different populations: native valve IE in intravenous drug users (IVDU), native valve IE in non-IVDU, prosthetic valve IE, and IE on a cardiac device.

Site of IE acquisition was defined following ICE recommendations [17]. In brief, community-acquired IE was defined as IE diagnosed within the first 48 hours of admission in a patient who did not fulfill the criteria for nosocomial or health care–associated infection. Nosocomial IE was defined as IE in a patient who had been hospitalized for more than 48 hours before the onset of signs or symptoms consistent with IE. Health care–associated IE was an IE diagnosed within 48 hours of admission of an outpatient with any of the following criteria: intravenous therapy, wound care, or specialized nursing care at home within the 30 days before the onset of IE; attendance at a hospital or hemodialysis clinic or receipt of intravenous chemotherapy within the 30 days before the onset of IE; hospitalization in an acute care hospital for two or more days during the 90 days before the onset of IE; or residence in a nursing home or long-term care facility[69].

A cardiac device was defined as a permanent pacemaker and/or cardioverter-defibrillator.

Perivalvular extension was considered to be substantial when abscesses were present or other echocardiography findings suggested that the infection was invasive (communication between chambers, wall dissection, or large valvular dehiscence). Prosthetic valve IE was defined as infection occurring on any type of non-native tissue or mechanical device. The EuroSCORE20 was used to assess operative risk [70, 71].

We used the Charlson comorbidity index as a method of estimating the risk of death from comorbid disease [72].

Chronic immunosuppressive therapy was defined as the administration of recognized immunosuppressive agents for more than 30 days at the time of IE diagnosis.

CNS event defined as an acute neurological deficit of vascular etiology lasting more than 24 hours [73]. Systemic embolization was defined as an embolic event outside of the central nervous system. Congestive heart failure was defined according to the New York Heart Association classification system [74].

Intra-cardiac complications were based on echocardiography findings (communication between chambers, wall dissection abscess or dehiscence).

OBJECTIVES

I. To assess the current situation of IE in a whole country.

1.1 To describe the changes in epidemiology and clinical manifestations of IE in a nationwide study.

1.2 To evaluate the risk factors of early and late mortality of IE during the first five years of the study.

II. To evaluate the epidemiological, microbiological, and clinical characteristics of Enterococcal IE

2.1 To identify characteristics that could help to identify IE among patients with E-BSI

2.2 To compared the outcome of E-BSI in patients with and without IE in order to assess the potential consequences of misdiagnosis.

2.3 To validate the NOVA score as a model for predicting patients with enterococcal bloodstream infection at low risk for IE.

III. To evaluate the systematic performance of new diagnostic imaging techniques in IE patients.

3.1 To evaluate the clinical impact of systematic whole-body 18F-FDG PET-CT (PET-CT) for the diagnosis of septic embolisms in patients with IE.

MATERIAL, METHODS AND RESULTS

I. Current situation of Infective Endocarditis in Spain

The study sample comprised all consecutive patients with IE in the 25 centers from January 1st, 2008 to December 31st, 2012. GAMES consists of multidisciplinary groups dedicated to improving the management of IE. These hospital-based endocarditis groups include microbiologists, infectious disease physicians, heart surgeons, imaging and clinical, cardiologists. Centers without cardiac surgery unit had to be able to follow patients referred elsewhere for surgery. These groups prospectively recorded all consecutive episodes of IE at their institutions and collected the data according to a pre-established clinical form with common standard definitions. [10-12] At discharge, the clinical forms were sent to the coordinating center or data were entered directly by the investigators through a secure data entry system. In the coordinating center, specialized clinicians and data managers reviewed the data for accuracy and contacted the referring centers, if necessary, for queries and clarifications. Enrollment began in January 2008. This report includes data from the first 1804 consecutive cases collected. Patients were followed for one year.

Because of the differences in clinical setting and outcome, we classified IE episodes as follows: native valve in non-IVDU, native valve in IVDU, prosthetic valve and IE involving cardiac devices.

In order to provide practical information to clinicians, the four classic types of IE were compared: native valve IE in IVDU and in non-IVDU, prosthetic valve IE, and IE affecting intra-cardiac devices.

Statistical analysis

Quantitative variables were expressed as mean ± SD or as medians with IQR, as appropriate; qualitative variables were expressed as frequency and percentage. Continuous variables were compared using the t test, and categorical variables were compared using the chi-square test or Fisher exact test when the chi-square test was not appropriate. Adjusted odds ratios were computed using logistic regression analysis. Stepwise logistic regression analysis was performed including variables with a p value ≤0.1 in the univariate analysis. All statistical analyses were performed using SPSS software version 18 (IBM PASW Statistics 18.0, Armonk, New York, USA).

Incidence of IE

The 25 centres that participated in this study are located throughout Spain and attend an estimated population of 10,218,634 habitants, ie, 21.7% of the Spanish population [75]. Therefore, we estimated an annual incidence of at least 3.5 cases of IE per 100,000 inhabitants.

General characteristics of the cohort

From January 2008 to December 2012, a total of 1804 cases were diagnosed. Patients surviving the initial admission were followed for one year. Enrolment from the participating centers was widely distributed. The median age of the cohort was 69 years (IQR, 55-77; mean, 65.1), and 1228 (68.0%) patients were male. The most common underlying conditions are summarized in table 1. The main conditions were previous cardiac surgery (620, 34.4%); heart failure (531, 29.4%), and diabetes mellitus (471, 26.1%). Other comorbidities that were not as frequent but nevertheless had a high impact on clinical course were previous IE episode (126, 6.9%), hemodialysis (79, 4.4%), HIV infection (39, 2.2%), and transplantation (27, 1.5%). The mean Charlson-age corrected comorbidity Index was 4.49±2.6. The most common predisposing conditions were degenerative native valve disease (41.8%) followed by prosthetic valves (29%), rheumatic valve disease (8.0%), intravenous catheter (7.5%), and congenital heart disease (3.2%).

Table 1. Epidemiological and clinical characteristics of 1804 episodes of infective endocarditis prospectively collected in Spain.

	TOTAL N=1804	**Native non-IVDU N=1079**	**Native IVDU N=52**	**Prosthetic N=504**	**Device N=169**	**p**
Median age (IQR)	69 (57 - 77)	68.7 (55-77)	39.9 (33.–45)	71.1 (61 - 77)	71.4 (60-78)	<.01
Male (%)	1228 (68.1)	728 (67.5)	42 (82.4)	337 (67.0)	121 (71.6)	0.10
Underlyingconditions						
Heartfailure	531 (29.4)	**252 (23.6)**	0	**203 (40.7)**	76 (46.3)	<.01
Previouscardiacsurgery	620 (34.4)	95 (8.9)	**1 (2.0)**	488 (97.2)	36 (21.4)	<.01
Diabetes mellitus	471 (26.1)	289 (26.8)	**1 (1.9)**	131 (26.0)	50 (29.8)	<.01
Mild renal insufficiency	188 (10.4)	88 (8.2)	**1 (2.0)**	70 (14.0)	**29 (17.3)**	<.01
Severe renal insufficiency	280 (15.5)	168 (15.7)	5 (9.8)	73 (14.5)	34 (20.1)	.22
Atrial fibrillation	457 (25.3)	190 (17.9)	0	**219 (44.4)**	48 (29.1)	<.01
Lungdisease	312 (17.3)	189 (18.0)	**2 (3.9)**	90 (18.2)	31 (18.9)	.07
Neoplasm	290 (16.1)	203 (18.9)	0	69 (13.7)	18 (10.7)	<.01
HIV infection	39 (2.2)	13 (1.2)	**23 (44.2)**	3 (0.6)	0	<.01
Previous IE	126 (7.0)	43 (4.0)	6 (11.5)	64 (12.8)	13 (7.8)	<.01
Charlson-ageindex	4.49 (2.6)	4.46	2.92	4.57	4.92	<.01

	TOTAL N=1804	Native non-IVDU N=1079	Native IVDU N=52	Prosthetic N=504	Device N=169	p
Transferredfromother hospital	479 (26.6)	**281 (26.0)**	13 (25.0)	**139 (27.6**	**46 (27.2)**	.91
Symptoms before admission (median days; IQR)	21 (7-60)	14 (5-55)	7 (4-21)	7 (3-21)	8 (5-60)	.58
Mitral	808 (44.8)	**579 (53.7)**	14 (26.9)	**211 (41.9)**	4 (2.4)	<.01
Tricuspid	99 (5.5)	58 (5.4)	24 (46.2)	4 (0.8)	**13 (7.7**	<.01
Pulmonary	29 (1.6)	16 (1.5)	3 (5.8)	8 (1.6)	2 (1.2)	.11
Site of acquisition						
Nosocomial	507 (28.1)	241 (23.1)	1 (1.9)	190 (39.7)	75 (46.9)	<.01
Community-acquired	1061 (58.8)	701 (67.3)	49 (94.2)	251 (52.6)	60 (37.7)	<.01
HCR	162 (9.0)	100 (9.6)	2 (3.8)	36 (7.5)	24 (15.1)	.01
Presentation						
Fever>38°C	1506 (83.4)	899 (84.0)	43 (84.3)	427 (85.9)	137 (82.0)	.01
Splinterhemorrhages	41 (2.3)	34 (11.0)	4 (16.0)	3 (2.7)	-	.01
Oslernodes	35 (1.9)	27 (8.4)	2 (7.7)	6 (5.0)	-	.38
Janewaylesions	43 (2.4)	29 (9.5)	4 (16.0)	9 (8.0)	1 (6.3)	.63
Roth spots	18 (1.0)	13 (4.5)	1 (4.0)	4 (3.8)	-	.84
Splenomegaly	209 (11.6)	140 (13.7)	16 (31.4)	46 (9.5)	7 (4.3)	<0.01
New murmur	577 (32.0)	430 (44.8)	23 (50.0)	115 (26.4)	9 (5.7)	<0.01
Worsening of oldmurmur	221 (12.3)	138 (16.3)	2 (4.7)	74 (18.4)	7 (4.5)	<0.01
Mean CRP (SD)	61.5 (87.2)	65.8 (90.2)	37.2 (68.0)	55.2 (84.9)	60.9 (79.1)	.015
Elevated RF	160 (8.9)	104 (25.8)	5 (26.3)	33 (17.8)	18 (26.1)	.18

CRP,C reactive protein; **IE**, infective endocarditis; **HCR**, health care–related; **RF**, rheumatoid factor.

Affected valve. Most of the patients (62.7%) had native valve IE, and most episodes were left-sided (mitral 808 [44.8%] and aortic valves 852 [47.2%]). The tricuspid valve was involved in 99 cases (5.5%) and the pulmonary valve in 29 cases (1.6%). Prosthetic valve endocarditis occurred in 504 cases (27.9%) and device-related endocarditis in 169 patients (9.3%).

The site of acquisition was determined in 95.9% of patients (Table 1); 28.1% episodes were classified as nosocomial. In total, 479 patients (26.6%) were transferred from another hospital. In the case of community-acquired episodes, most patients (86%) were admitted within 1 month of the initial signs of illness (12.6% at 1-3 months and 6.3% >3 months).

Clinical manifestations are listed in table 1. It is noteworthy that the classic signs of IE were uncommon. These included splenomegaly (11.6%), splinter hemorrhages (2.3%), Janeway spots (2.4%), and Osler nodes (1.9%). However, patients with IE had other common manifestations (respiratory [41%], renal [39%], neurological [19.7%], osteoarticular [11.5%], and ocular [6.3%]).

Etiology. Most episodes (78.8%) were caused by Gram-positive microorganisms, followed by Gram-negative microorganisms (5.2%), fungi (2.4%), anaerobes (1.2%), and polymicrobial infections (1.9%). The distribution of the most common microorganisms is shown in table 2. Twenty-two episodes were caused by microorganisms of the HACEK

group. Other fastidious microorganisms included *Coxiella burnetii* (15), *Listeria monocytogenes* (6), *Tropheryma whipplei* (5), *Bartonella* spp (4), and *Brucella melitensis* (1). Accordingly, the rate of unknown etiology of endocarditis was 9.1%.

Diagnosis. Blood cultures were obtained in 1787 patients (99.1%) and provided the etiology in 1523 (85.3%). Of the 264 patients (14.7%) with negative blood cultures, 34% had received antimicrobial agents in the previous week. An etiologic diagnosis was achieved in 106 cases with a combination of the following techniques: heart valve PCR (34; 20.7%), serology (99; 60.3%), and extracardiac cultures (59; 35.9%).

Transesophageal echocardiography was used in most patients (76.3%), and 1148 (83.4%) presented vegetations. Abscess was the most common paravalvular complication (27.8% of patients), whereas 26.6% of patients with prosthetic valve IE had evidence of a prosthetic valve complication such as dehiscence or new paravalvular regurgitation.

Comparison of the four types of IE

Overall, IE episodes were classified as follows: native valve in non-IVDU (n=1079), native valve in IVDU (n=52), prosthetic valve (n=504), and IE involving cardiac devices (n=169) (Tables 1 and 2).

Table 2. Etiology, diagnosis, and outcome of 1,804 episodes of infective endocarditis prospectively collected in Spain.

	TOTAL N=1804	Native non-IVDU N=1079	Native IVDU N=52	Prosthetic N=504	Device N=169	p
Definite IE	1498 (83.0)	919 (85.6)	48 (92.3)	409 (81.3)	122 (72.2)	<0.01
Possible IE	300 (16.6)	155 (14.4)	4 (7.7)	94 (18.7)	47 (27.8)	<0.01
Etiology						
*Staphylococcus*spp	728 (40.3)	382 (35.3)	30 (55.8)	218 (43.2)	98 (58.0)	<0.01
S. aureus	426 (23.6)	**278 (25.8)**	**26 (50.0)**	77 (15.3)	**45 (26.6)**	<0.01
MRSA	66 (3.7)	43 (4.0)	2 (3.8)	13 (2.6)	8 (4.7)	.46
CNS	302 (16.7)	104 (9.7)	4 (7.7)	**141 (28.0)**	**53 (31.5)**	<0.01
*Streptococcus*spp.	440 (24.4)	**329 (30.5)**	8 (15.4)	86 (17.1)	17 (10.1)	<0.01
S. bovis	117 (6.4)	80 (7.4)	0	32 (6.5)	5 (3.0)	0.036
S. viridans	223 (12.3)	171 (16.0)	7 (13.5)	38 (7.5)	7 (4.1)	<0.01
Others	100 (5.5)	79 (7.3)	1 (1.9)	15 (3.0)	5 (5.3)	0.001
*Enterococcus*spp.	230 (12.7)	**142 (13.2)**	5 (9.6)	**77 (15.3)**	6 (3.6)	.001
Other Gram +	26 (1.4)	14 (1.3)	2 (3.8)	8 (1.5)	2 (1.1)	.48
Gram -	93 (5.2)	53 (4.9)	-	25 (5.0)	15 (8.9)	0.05
Fungi	44 (2.4)	21 (1.9)	2 (3.8)	15 (3.0)	6 (3.6)	.38
Negative BC	164 (9.1)	**100 (9.3)**	5 (9.6)	**48 (9.5)**	**11 (6.5)**	.67
Vegetations	1284 (71.2)	836 (77.6)	41 (78.8)	296 (58.8)	111 (65.7)	<0.01
Intracardiaccomplication	501 (27.8)	309 (28.9)	9 (17.6)	171 (33.9)	12 (7.1)	<0.01
TEE	1377 (76.3)	776 (72.0)	23 (44.2)	450 (89.3)	128 (76.2)	<0.01
Clinicalcourse						
Embolisms	525 (29.1)	322 (30.5)	30 (60.0)	143 (28.9)	30 (18.2)	<0.01
New heartfailure	698 (38.7)	488 (45.9)	15 (29.4)	214 (43.2)	32 (19.2)	<0.01
Persistent BSI	151 (8.4)	90 (8.6)	1 (2.0)	45 (9.2)	15 (9.0)	.37

	TOTAL N=1804	Native non-IVDU N=1079	Native IVDU N=52	Prosthetic N=504	Device N=169	p
Surgery						
Indicated (%)	1152 (63.9)	661 (61.4)	25 (48.1)	341 (67.7)	125 (74.0)	<0.01
Performed (%)	797 (44.2)	452 (41.9)	15 (28.8)	220 (43.8)	110 (65.1)	<0.01
Reasonsforsurgery						
Cardiacinsufficiency	373 (20.7)	257 (25.9)	6 (13.0)	104 (22.3)	6 (3.7)	<0.01
Earlyprosthetic IE	78 (4.3)	8 (0.8)	1 (2.2)	67 (14.4)	2 (1.2)	<0.01
Late prosthetic IE	78 (4.3)	1 (0.1)	-	74 (15.9)	3 (1.8)	<0.01
Valvular insuf.	315 (17.5)	225 (22.7)	11 (23.9)	78 (16.8)	1 (0.6)	<0.01
Median hospital stay (IQR)	36 (21-53)	36 (21-51)	36 (22-48)	39 (18–54)	34 (23-53)	.92
In-hospital mortality (%)	521 (28.9)	301 (27.9)	8 (15.4)	184 (36.5)	28 (16.6)	<0.01
One-yearmortality (%)	116 (9.1)	81 (10.4)	1 (2.27)	29 (9.0)	5 (3.5)	.05

BC, blood cultures;**BSI**, Blood stream infection; **CNS**, coagulase-negative staphylococci; **TEE**, transesophageal echocardiogram;

IE, infective endocarditis; **IQR**, interquartile range; **MRSA**, methicillin-resistant S. aureus

Non-IVDU patients with native valve IE

Most of the patients in our series (59.8%) were non-IVDU, which is therefore the most heterogeneous group. Although it is difficult to identify one characteristic that stands out, these patients presented less frequently history of heart failure (23.6%) or renal failure (23.9%). Most cases were community-acquired, and *Streptococcus* spp. was the most common pathogen involved.

Native valve IE in IVDU accounted for the smallest group of our series. Native valve IE affected significantly younger patients with fewer comorbid conditions, except in the case of HIV infection (44.2%). Acquisition was nosocomial in only 1.9% of the cases and, interestingly, half of these patients had left-sided IE. A clinical presentation was more evident in this population including splinter hemorrhages (16%) and splenomegaly (31.4%). *S. aureus* predominated as the etiological microorganism (53.8%), and embolisms were frequent (60.0%). Outcome was clearly better in this group.

Prosthetic valve IE.

The highest in-hospital mortality was recorded in patients with prosthetic valve IE (36.5% $p<0.01$); however, it is of even greater concern that infection was nosocomial in 39.7% of these patients.

Coagulase-negative staphylococci accounted for 28.0% of the cases. Accordingly, intra-cardiac complications were significantly more frequent (33.9%).

Cardiac device IE.Patients with heart devices were older, with highest comorbidity index and a great part (62%) were nosocomial or health care related. The device involved was surgically removed in 65.1% of the cases.

Short-term and long-term risk factors for mortality

Table 3 shows a comparison of the patients who survived (71.1%) and those who died (28.9%) during admission; table 4 shows the independent risk factors associated with a higher risk of in-hospital death. Independent mortality risk factors could be grouped as: epidemiological characteristics of the patient, endocarditis etiology (*Staphylococcus* spp [OR, 2.34], fungi [OR, 3.12]) and complications (intra-cardiac complication [OR, 1.67], heart failure [OR, 2.97], and septic shock [OR, 5.18]).

Table 3. Risk factors for in-hospital mortality

	Alive N=1283	**Dead N=521**	**p**
Median age (IQR)	67.6 (53.7 – 76)	73 (62.9 – 78.9)	<0.01
Male	907 (70.7)	321 (61.8)	<0.01
Underlying condition			
Heart failure	326 (25.6)	205 (40.0)	<0.01
Previous cardiac surgery	411 (32.4)	209 (40.4)	.001
Diabetes mellitus	294 (22.9)	177 (34.1)	<0.01
Mild renal insufficiency	117 (9.2)	71 (13.8)	.004
Severerenal insufficiency	163 (12.8)	117 (22.7)	<0.01
Atrial fibrillation	288 (22.8)	169 (33.2)	<0.01
Lung disease	209 (16.7)	103 (20.4)	.067
Neoplasm	199 (15.5)	91 (17.5)	.296
HIV infection	29 (2.3)	10 (2.0)	.675
Previous IE	101 (7.9)	25 (4.8)	.020
Charlson-age comorbidity (SD)	4.1 (2.5)	5.4 (2.5)	<0.01
Transferred from other hospital	340 (26.5)	139 (26.7)	.938
Symptoms before admission (median days [IQR])	6 (1 – 18)	2 (0 – 8)	<0.01
Affected valve			
Aortic	594 (46.3)	258 (49.5)	.214
Mitral	541 (42.2)	267 (51.2)	<0.01
Tricuspid	80 (6.2)	19 (3.6)	.029
Pulmonary	22 (1.7)	7 (1.3)	.570

	Alive N=1283	**Dead N=521**	**p**
Proven endocarditis	1054 (82.3)	444 (85.9)	.064
Possible endocarditis	227 (17.7)	73 (14.1)	.064
Etiology (%)			
S. aureus	237 (18.5)	189 (36.5)	<0.01
Methicillin-resistant*Staphylococcus aureus*	34 (2.7)	32 (6.1)	<0.01
Coagulase-negativestaphylococci	201 (15.7)	101 (19.4)	.058
*Streptococcus*spp.	371 (28.9)	69 (13.2)	<0.01
Enterococcus spp.	177 (13.8)	53 (10.2)	.037
OtherGram-positivemicroorganisms	19 (1.5)	7 (1.3)	.824
Gram-negativemicroorganisms	76 (5.9)	17 (3.3)	.021
Fungi	25 (2.0)	19 (3.6)	.034
Negative blood cultures	110 (8.6)	54 (10.4)	.230
Vegetation	887 (69.1)	397 (76.5)	.002
Intracardiac complication	310 (24.3)	191 (37.0)	<0.01
Transesophageal echocardiogram	1004 (78.3)	375 (71.9)	<0.01
Persistent bacteremia	69 (5.5)	82 (16.7)	<0.01
Heart surgery			
Indicated	728 (56.8)	424 (81.4)	<0.01
Operated on	598 (46.7)	199 (38.2)	.001
Reasons for surgery (%)			
Cardiac insufficiency	254 (21.5)	119 (24.5)	.185
Early prosthetic IE	47 (4.0)	31 (6.4)	.036
Late prosthetic IE	46 (3.9)	32 (6.6)	.019
Valvular insufficiency	245 (20.8)	70 (14.4)	.003
Median hospital stay (IQR)	41 (27 – 55)	23 (11 – 42)	<0.01

BC,blood cultures; **TEE,** transesophageal echocardiogram; **IE**,infective endocarditis**; IQR**, interquartile range; **SD**,standard deviation.

Table 4. Independent risk factors for in-hospital mortality

Factor	OR	95% CI	p
Age	1.02	1.01 – 1.03	<0.01
Immunosuppressive therapy	2.61	1.68 – 4.04	<0.01
Previous heart surgery (previous to the episode of IE)	1.53	1.17 – 2.00	.002
CNS event	2.47	1.91 – 3.19	<0.01
Atrial fibrillation	1.45	1.09 – 1.93	.011
Staphylococcus aureus	2.34	1.75 – 3.12	<0.01
Fungi	3.12	1.50 – 6.49	.002
Intra-cardiac complication	1.67	1.30 – 2.14	<0.01
Heart failure	2.97	2.30 – 3.83	<0.01
Septic shock	5.18	3.62 – 7.40	<0.01

Overall, 1283 patients survived hospital admission, and one-year follow-up was available in 1035 (80.6%). Independent risk factors for one-year mortality are shown in table 5 and include increasing age (OR, 1.02), neoplasm (OR, 2.46), renal insufficiency (OR, 1.59), and heart failure (OR, 4.42). Surgery was independently associated with a decreased risk of one-year mortality (OR, 0.44) and was the only factor amenable of intervention.

Table 5. Independent risk factors for one-year mortality

Factor	OR	95% CI	p
Age	1.02	1.00 - 1.03	.005
Neoplasm	2.46	1.57 – 3.86	<0.01
Surgery	0.44	0.286 - 0.694	<0.01
Renal insufficiency	1.59	1.04 – 2.42	.030
Heart failure	4.42	1.06 - 18.40	.041

II. Epidemiological, microbiological, and clinical characteristics of Enterococcal IE.

Our design includes 2 studies. In the first one, we aimed to assess the frequency of enterococcal IE by analyzing a prospective cohort including all patients with E-BSI. In the second, we performed a case-control study comparing patients with and without enterococcal IE. Both studies were performed in a 1550-bed tertiary center attending a population of 715,000 habitants.

Prospective cohort study: The study sample comprised all cases of E-BSI diagnosed in our institution from September 2003 to October 2012. During this period, we identified 2 phases that differed with respect to the diagnosis of IE. From 2003 to 2007, patients with E-BSI were managed by the attending physician who requested consultation with the infectious diseases department or the laboratory of echocardiography [3] according to his/her own criteria (Period A). From 2008-2012 (Period B), a physician from the infectious diseases department visited the patients with E-BSI and promoted the systematic use of echocardiography. We recommended the systematic performance of TEE in most patients, provided the patient consented to and the attending physician agreed with the indication. The need for TEE in patients referred for transthoracic

echocardiography (TTE) was occasionally indicated by the cardiologist. Accordingly, in some patients, only TTE was performed, in some TTE and TEE, and in others neither of the 2 techniques. From September 2003 onward, clinical data on all patients with enterococcal IE were collected prospectively as part of a pre-established protocol.

Case-control study: We designed a case-control study to identify a subgroup of patients at very low risk of enterococcal IE in whom systematic TEE could safely be deemed unnecessary. All patients fulfilling the modified Duke criteria [12] for IE were considered cases, and patients with E-BSI and a TEE result that ruled out IE were considered controls. Control patients were randomly selected from among patients with E-BSI and a negative TEE result and no criteria for IE according to the modified Duke criteria [12].Both groups were independently selected from the period in which they presented.

To evaluate the possibility of misdiagnosed IE we reviewed the clinical records of a randomly selected significant sample (176/1127) of patients with enterococcal bacteremia that did not undergo TEE. We analyzed main clinical characteristics and duration of therapy. The selected parameters were: previous valve

disease; origin of bacteremia; number of positive blood cultures; recurrence of bacteremia; clinical, microbiological, and/or radiological findings suggestive of septic embolism; NOVA score; treatment and outcome during admission and follow-up. Patients were followed up for a mean of 653 days after discharge.

Statistical analysis

In the descriptive study, qualitative variables are presented as percentages with their confidence interval (CI) and quantitative variables as the mean and CI and/or median with the interquartile range, depending on the distribution. Clinical and microbiological variables were studied to obtain a predictive model for enterococcal endocarditis. Differences between groups were analysed using the t test, median test, X2 test, or Fisher exact test, depending on the characteristics of the variables and their distribution between groups. The sensitivity of TTE/TEE was compared with a McNemar test for pair samples. The evolution of the variables during the study period was assessed using the autoregressive integrated moving average test.

In order to develop a reliable algorithm that made it possible to rule out the need for TEE, we designed a strategy based on bootstrapping. Given that the same case-control dataset was used for development of the model, testing, assessment of goodness of fit,

and establishing threshold values, we implemented bootstrapping techniques to avoid overfitting. The association between individual predictors and the risk of IE was assessed using binary multivariate logistic regression including variables selected from the exploratory univariate analysis. Final variables in the model were selected using a backward stepwise approach based on Alkaike's information criterion [16] and clinical judgment. This logistic regression model was validated by 2 runs of 2000 bootstrap replications [17] with IE prevalence values of 50% (as in the case-control group) and 4.3% (as in the prospective cohort). After validation, we developed a quantitative score for the risk of endocarditis by rounding the estimated odds ratio (OR) values of the model. This synthetic univariate prediction score was tested in a second logistic regression model and again validated by 2 bootstrapp runs, as described above. Additionally, the logistic regression model was calibrated by plotting predicted versus observed probabilities. Finally, bootstrap-based 95% confidence intervals were obtained for sensitivity and specificity and overlaid on the ROC plot [18, 19]. A conservative cut-off for the predictive score was based on the maximization of sensitivity, as recommended for screening methods. Data were analyzed using SPSS, version 18.0 (SPSS Inc, Chicago, Illinois, USA) and R[20].

Incidence of enterococcal endocarditis

During the study period (2003-2012), we detected 1515 episodes of E-BSI. The annual distribution is shown in table 6. Of these, 679 (2.1 episodes/1000 admissions) occurred in period A (2003-2007) and 836 (3.1 episodes/1000 admissions) in period B (2008-2012). This increase was statistically significant (p<0.001). The annual increase in E-BSI was 0.167 episodes/1000 admissions (95%CI, 0.100-0.234; P<0.001). Overall, 388 patients underwent TEE after the episode of E-BSI: 100 during period A (14.7% of all E-BSI) and 288 during period B (34.4% of all E-BSI).

Table 6. Enterococcal bloodstream infections (E-BSI) and endocarditis (EE) during the study period.

Year	E-BSI episodes	E-BSI/1,000 admissions	EE episodes	EE/E-BSI (%)	EE / 1000 admissions
2003	109	2.0	2	1.8%	0.04
2004	114	1.9	8	7.0%	0.13
2005	129	2.1	3	2.3%	0.05
2006	149	2.3	5	3.3%	0.08
2007	178	2.6	8	4.4%	0.12
2008	177	3.0	5	2.8%	0.12
2009	170	3.2	9	5.2%	0.15
2010	149	2.7	3	2.0%	0.05
2011	181	3.4	14	7.7%	0.26
2012	159	3.2	8	5.0%	0.14

Enterococcal IE was detected in 65 patients, who accounted for 4.29% of all patients with E-BSI (3.76% in period A and 4.54% in period B). The increase in the annual incidence of enterococcal IE was 0.012 episodes/1000 admissions (95%CI, 0.004-0.020; P=0.004). IE was diagnosed in 16.7% of patients who underwent TTE and 35.5% of the patients who underwent TEE. Of all the episodes of enterococcal IE, only 18 cases (27.7%) were detected by TTE; the remaining 47 (72.3%) were demonstrated only after TEE. Sensitivity of TTE and TEE for the diagnosis of enterococcal IE was 32% vs 95% (p<0.01).

Comparison of E-BSI patients with and without endocarditis

In order to identify characteristics that could help to identify IE among patients with E-BSI, the 65 cases were compared with the 65 controls. The epidemiological, microbiological, and clinical characteristics of both groups are shown in table 7. No differences were detected in age or sex, but patients with IE more frequently presented a history of stroke (27.7% vs 13.8%, p=0.05), immunosuppressive therapy (24.2% vs 10.8%, p=0.03), previous heart valve disease (63.0% vs 29.2%, p<0.01), and previous heart valve surgery (44.6% vs 24.6%, p=0.03). Malignancy, however, was more frequent in controls (23% vs 41.5%, p=0.02).

Table 7. Epidemiological, clinical and microbiological characteristics of patients with and without enterococcal endocarditis.

	Endocarditis (%)	**No Endocarditis (%)**	**p**
Mean age (SD)	71.2 (11.3)	70.3 (13.6)	0.7
Females **Males**	18 (27.7) 47 (72.3)	23 (35.4) 42 (64.6)	0.34
Underlying disease			
Congestive heart failure Yes No	 27(41.5) 38 (58.5)	 19 (29.2) 46 (70.8)	 0.09
Stroke Yes No	 **18 (27.7)** 47 (72.3)	 **9 (13.8)** 56 (86.2)	 **0.05**
Transplant Yes No	 6 (9.2) 59 (90.8)	 7 (10.8) 58 (89.2)	 0.77
Immunosuppression Yes No	 **15 (23)** 50 (76.9)	 **7 (10.8)** 58 (89.2)	 **0.03**
Neoplasm Yes No	 **14 (21.5)** 51 (78.5)	 **27 (41.5)** 38 (58.5)	 **0.02**
Renal failure Yes No	 23 (35.4) 42 (64.6)	 21 (32.3) 44 (67.7)	 0.41
Previous endocarditis Yes No	 8 (12.3) 57 (87.7)	 4 (6.2) 61 (93.8)	 0.17
Heart valve disease Yes No	 **41(63.1)** 24 (36.9)	 **19 (29.2)** 46 (70.8)	 **<0.01**
Prostheticvalve Yes No	 31(47.7) 34 (52.3)	 17 (26.2) 48 (73.8)	 0.18
Native valve disease Yes No	 10 (15.4) 55 (84.6)	 2 (3.1) 63 (96.9)	 0.07
Previous cardiac valve surgery Yes No	 25 (38.5) 40 (61.5)	 16 (24.6) 49 (75.4)	 0.03
Mean Charlsonindex (SD)	**5.47 (2.3)**	**6.5 (2.9)**	**0.02**
Clinical presentation			
Fever Yes No	 **59 (90.7)** 6 (9.2)	 **58 (89.2)** 7 (10.8)	 **0.36**
Heart murmur Yes No	 **37 (56.9)** 28 (43.1)	 **19 (29.2)** 46 (70.8)	 **<0.01**
Etiology			
E. faecalis Yes No	 **56 (86.2)** 9 (13.8)	 **38 (58.5)** 27 (41.5)	 **<0.01**

E. faecium			
Yes	7 (10.8)	24 (36.9)	<0.01
No	58 (89.2)	41 (63.1)	
Enterococcus. spp			
Yes	2 (3.1)	3 (4.6)	0.65
No	63 (96.9)	62 (95.4)	
Continuousbacteremia			
Yes	**61 (93.8)**	**45 (69.2)**	**<0.01**
No	4 (6.2)	20 (30.8)	
Site of acquisition			
Community			
Yes	**28 (43.1)**	**13 (20)**	**<0.01**
No	37 (56.9)	52 (80)	
Nosocomial			
Yes	30 (46.2)	45 (69.2)	0.01
No	35 (53.8)	20 (30.8)	
Health care–associated			
Yes	7 (10.8)	7 (10.8)	0.03
No	58 (89.2)	58 (89.2)	
Source of BSI			
Gastrointestinal			
Yes	9 (13.8)	31 (47.7)	<0.01
No	56 (86.2)	34 (52.3)	
Unknown*			
Yes	**25 (38.5)**	**7 (10.8)**	**<0.01**
No	40 (61.5)	58 (89.2)	

* Overral 16/32 (50%) had colonoscopy (4/7 patients without endocarditis an 12/25 patients with endocarditis).

Episodes of IE were caused mainly by E. faecalis (86.2% vs 58.5%, p<0.01). In addition, they were associated with continuous bacteremia (93.8% vs 69.2%, p<0.01), community acquisition (43.1% vs 20%, p<0.01), and unknown source of infection (38.4% vs 10.7%, p<0.01). In the control group, however, E-BSI was mainly nosocomial (69.2% vs 46.2%, p=0.01) and had a gastrointestinal origin (48.4% vs 13.8%, p<0.01).

As for outcome, patients with IE presented more complications (Table 8) and had significantly higher mortality (38.4% vs 15.4%, p<0.01).

Table 8. Outcome of patients with and without endocarditis.

	Endocarditis N=65 (%)	**No Endocarditis N=65 (%)**	**p**
Complications			
Cardiacfailure	**23(35.9)**	**6 (9.2)**	**<0.01**
Persistent BSI	13 (21.7)	9 (13.8)	0.09
CNS vascular event	**5 (7.9)**	**0**	**0.02**
Other than CNS embolic event	**10 (15.9)**	**2 (3.1)**	**0.007**
Treatment			
Empiricadequatetreatment	**54 (96.4)**	**13 (20.0)**	**<0.01**
Mean days of overall treatment (SD)	**34 (17.1)**	**15 (8.2)**	**<0.01**
Death	**25 (38.4)**	**10 (15.4)**	**<0.01**
Mean days of hospital stay (SD)	**47.3 (29.7)**	**36.2 (33.0)**	**0.04**

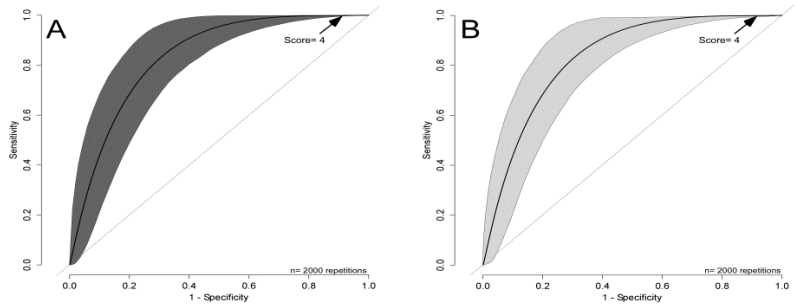
BSI, bloodstream infection; CNS, central nervous system.

Score for identifying bacteremic patients with a low risk of IE

The multivariate analysis showed that enterococcal IE is 9-fold more probable in patients with positive blood cultures in all of 3 blood cultures or the majority of more than 3 blood cultures (OR, 9.9; 95%CI 2.2-40.6). Other factors independently associated with enterococcal IE were a history of heart valve disease (OR, 3.7; 95%CI, 1.6-8.7) and an unknown source of bacteremia (OR, 7.7; 95%CI, 2.5-23.8). We developed a score using the variables selected in the multivariate model by including those that improved sensitivity and specificity for predicting enterococcal IE. This model validated both very well using bootstrap resampling based on prevalence values of 50% (slope

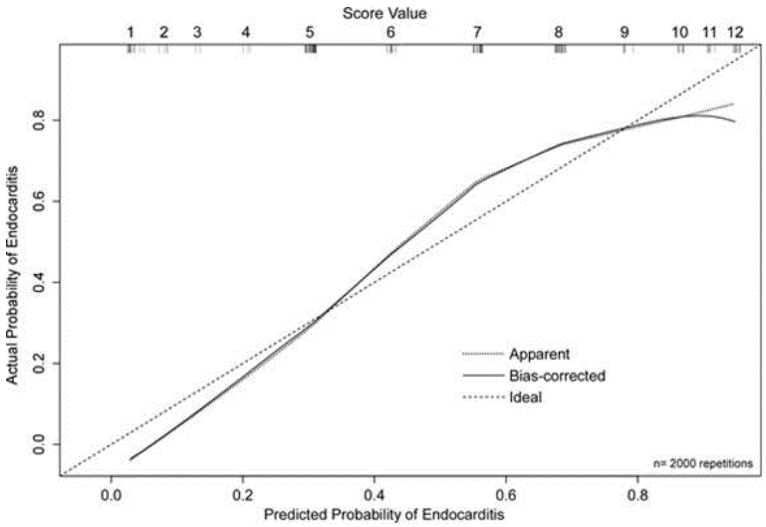
shrinkage factor for the agreement between the training and test samples, 0.88; maximum absolute error in predicted probability, 0.03) and 4.3% (slope shrinkage factor=0.90; maximum error=0.02). The ORs of these models were used to obtain representative weights by rounding to develop a synthetic score on a scale of 0 to 12 for the risk of IE in patients with E-BSI. The score, which we called the NOVA score, was based on the following variables: number of positive blood cultures (N) suggestive of continuous bacteremia (3/3 blood cultures or the majority if more than 3), 5 points;, 5 points; unknown origin of bacteremia (O), 4 points; prior valve disease (V), 2 points; and auscultation of a heart murmur (A), 1 point. The area under the ROC curve for the NOVA score was 0.829 (95%CI, 0.758-0.901). Again, this model was accurately validated by bootstrapping (slope shrinkage factor=0.99 and 1; maximum error=0.003 and 0.002, for the resampling runs each stratified by the IE prevalence values of 50% and 4.3%, respectively).The best binary cut-off value for ruling out IE without the need for TEE was established at a NOVA score <4 points (Figure 1).

Figure 1. Best binary cut-off value for ruling out IE without the need for TEE.



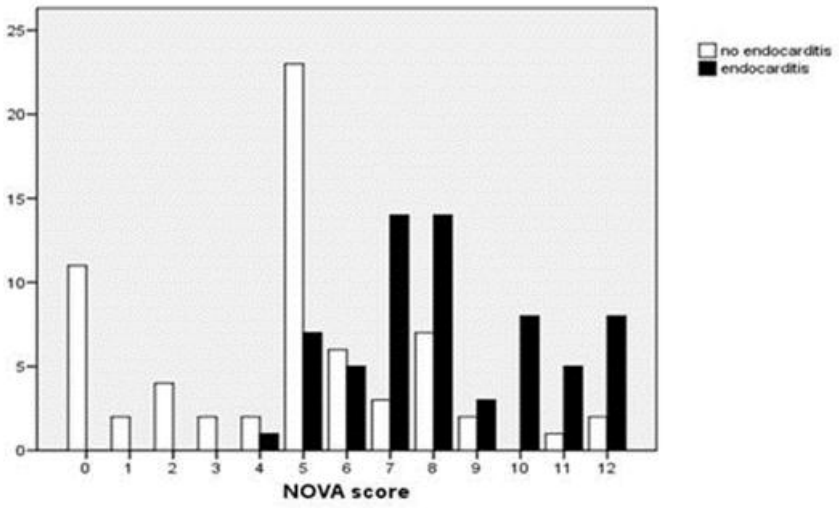
Using this cut-off, the model calibration curve excluded the risk of false negatives (Figure 2). The probability of enterococcal IE with different scores is as follows: 5 points, 23.3%; 6 points, 45.5%; 7 points, 82.4%; 8 points, 66.7%; 9 points, 60.0%; 10 points, 100%; 11 points, 83.3%; 12 points, 80%.

Figure 2. Model calibration curve



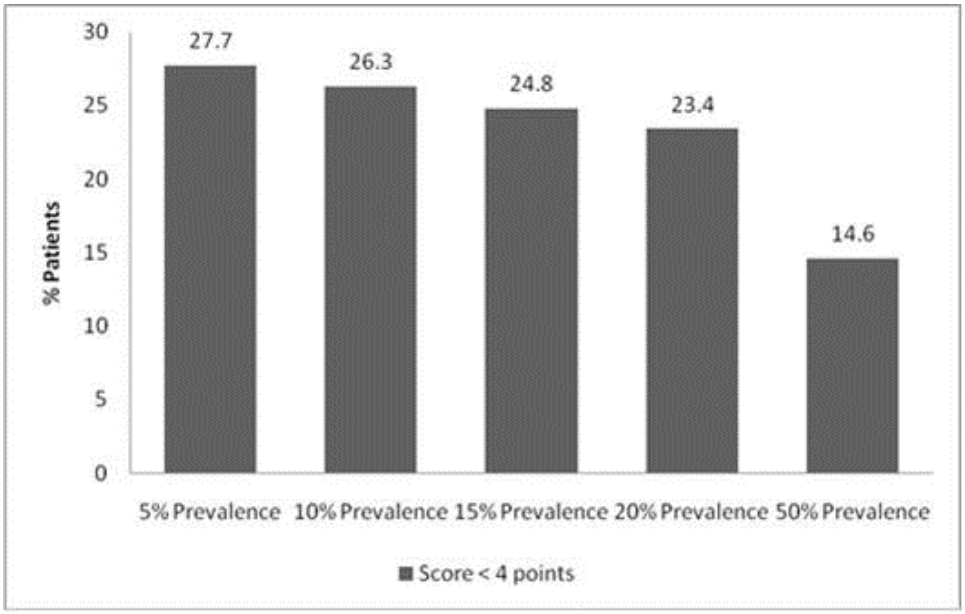
None of the 65 patients with enterococcal IE had a NOVA score lower than 4 in our series (Figure 3). According to this model, the percentage of patients with E-BSI who would not require echocardiography (score <4 points) ranged from 14.6% in a setting with a prevalence of endocarditis of 50%, such as our case-control study, to 27.7% in a setting with a 5% prevalence of endocarditis.

Figure 3. Distribution of Enterococcal IE and E-BSI, according to the NOVA score.



Therefore, according to our model, in populations with a low prevalence (5%) and high prevalence (20%) of IE, the proportion of patients with E-BSI in whom TEE may not be necessary is 27.7% and 23.4%, respectively (Figure 4).

Figure 4. Proportion of patients with E-BSI in whom TEE may not be necessary



Analysis of a sample of E-BSI patients who did not undergo echocardiography

After a careful record review, only 3/176 patients (1.70%) without TEE could have had IE according to the selected clinical criteria. All 3 had been treated for at least 4 weeks. Regarding length of treatment with appropriate regimens, only 12/176 patients received more than 2 weeks of therapy (3 with bacteremia of unknown origin, 6 with cholangitis, 1 with osteomyelitis, 1 with infected knee prosthesis, and 1 with fecal peritonitis). As for occurrence of embolic episodes, only 2 patients presented with clinical, microbiological, and/or radiological evidence of septic embolism: a 79-year-old patient with multiple bilateral pulmonary consolidations who refused to undergo TEE and a 92-year-old man with severe Alzheimer disease and L4-S1 osteomyelitis whose family refused TEE. Both patients had been treated at least 4 weeks before discharge. Finally, we classified the 176 patients according to the NOVA score. Overall, 106 had a score <4 points and were treated for a mean of 14 (SD 5.2) days. None of them presented clinical, microbiological, or radiological signs of embolism or IE during follow-up. Seventy patients had a NOVA score ≥4points. Three of these patients were thought to have had IE and 2 presented with an embolic complication, as previously mentioned. They had all been treated for more than 2 weeks. We cannot rule out the possibility of endocarditis in patients who died early after E-BSI.

External validation of the predictive NOVA score for infective endocarditis among patients with enterococcal bloodstream infection

Setting

S' OrsolaMalipighi Hospital in Bologna is a 1,420-bed tertiary teaching institution in Northern Italy, with approximately 72,000 hospital admissions per year. Institutional Ethics Committee approved the study.

To validate the NOVA score we performed a retrospective study of all the patients with E-BSI studied with echocardiography for ruling out IE. Using the records of our Laboratory of Microbiology all the patients with E-BSI, hospitalized at our hospital from January 2011 to December 2013, were identified. The patient charts were reviewed to assess if IE had been ruled out by echocardiography. Data of all the patients with echocardiography were collected in a case report form including the parameters of the NOVA score (Number of positive blood cultures, Origin of the bacteremia, previous Valve disease, and Auscultation of heart murmur). Clinical data up to 1 year after E-BSI were reviewed as follow-up.

The NOVA score was calculated for every enrolled patient considering the followings parameter and their values: number of blood cultures (3/3 or the majority if more than 3 = 5 points); origin of BSI (unknown= 4 points); prior valve disease (2 points); auscultation of heart murmurs (1 point).

Statistical analysis

Categorical variables were presented as absolute numbers and their relative frequencies. Quantitative variables were presented as mean and standard deviation (SD) if normally distributed or as median and interquartile range (IQR) if non-normally distributed.

The discriminatory power for the NOVA score was evaluated by the area under the receiver operating characteristic (ROC) curve. The Youden's J statistics was used to establish the optimal cutoff for discriminating patients at low and high risk for IE in our hospital. Sensibility, specificity, positive and negative predictive values were calculated according to the prevalence of enterococcal EI.

Results

During the study period, 449 patients with E-BSI were identified. Of them, 100 patients were studied with echocardiography: trans-thoracic in 83, trans-esophageal in 5, and both in 12 patients; and analyzed for this study.

Mean age was 64.7 (±15.8) years, 58% were male. The mean Charlson index was 4.3 (±1.8). E-BSI was classified as community acquired, health-care associated and hospital acquired in 19%, 23% and 58% of cases, respectively.

Etiological distribution of E-BSI was as follows: *E. faecalis* 61%, *E.faecium* 30%, and other *Enterococcus* spp. 9%.

Enterococcal IE was diagnosed in 24 patients, with a prevalence of 5.3% among all patients with E-BSI and 24% among those with an echocardiography.

Comparison of patients with and without IE is shown in Table 9.

Table 9. Patients with enterococcal bloodstream infection studied with echocardiography: comparison of patients with and without infective endocarditis

	Total, n=100 (100%)	**Patients without IE, N=76 (76%)**	**PatientswithIE, N=24 (24%)**	***P***
Demographic data				
Age (years) [mean (±SD)]	57 (±12)	64 (±16)	64 (±12)	0.9
Sex, male	56 (56)	40 (52)	16 (66)	0.2
Comorbidities				
Charlsonindex [mean (±SD)]	4.6 (± 2)	4.7 (±2)	4.3 (±1.8)	0.46
Chronickidneydisease	14 (14)	5 (18)	14 (70)	0.02
Neoplasia	34 (34)	30 (39)	4 (16)	0.49
Site of BSI acquisition				
Hospitalacquired	58 (58)	51 (67)	7 (29)	0.002
Healthcareassociated	23 (23)	11 (14)	12 (50)	0.001
Communityacquired	19 (19)	14 (18)	5 (20)	0.7
Source of BSI				
Unknown	47 (47)	23 (30.3)	24 (100)	<0.001
CVC	17 (17)	17 (22.3)	0	NA
Gastro-intestinal	14 (14)	14 (18.4)	0	NA
Biliary	10 (10)	10 (13.1)	0	NA
Urinary	8 (8)	8 (10.5)	0	NA
Skin and softtissues	4 (4)	4 (5.2)	0	NA
Diagnosticalprocedures				
TTE	95 (95)	75 (98)	20 (83)	0.01
TEE	17 (17)	3 (3)	15 (58)	<0.001
FDG-PET	20 (20)	8 (10)	12 (50)	<0.001
NOVA score				
Number of positive blood cultures [median (IQR)]	3 (2-3)	2 (2-3)	3 (3-4)	<0.001
Origin of BSI (unknown)	47 (47)	23 (30)	24 (100)	<0.001
Valve disease (previous)	31 (31)	16 (21)	15 (62)	<0.001
Auscultation of heart murmur	14 (14)	5 (6)	9 (37)	0.001
Endocarditis				
Mitral valve	17 (17)	0	17 (70)	NA
Aorticvalve	14 (14)	0	14 (58)	NA
Tricuspidvalve	2 (2)	0	2 (8)	NA
Pacemaker associated	2 (2)	0	2 (8)	NA

Abbreviations: BSI bloodstream infection; CVC central venous catheter; FDG-PET fluorodeoxyglucose-positron emission tomography; IQR interquartile range; NA not applicable; SD standard deviation; TTE transthoracic echocardiography; TEE transesophageal echocardiography.

The ROC curve analysis suggested that the NOVA score could acceptably discriminate low versus high risk patients, with an area under the curve of 0.96 (95%CI 0.93-0.99, p<0.00) (Figure 5). The probabilities of enterococcal IE for the different thresholds of the NOVA score were: 0%, 0-5 points (63 patients); 12.5%, 6-8 points (8 patients); 75%, 9-10 points (12 patients); and 82.3%, 11-12 points (17 patients) as reported. The optimal breakpoint in our cohort resulted to be >5.

Considering a pre-test prevalence of endocarditis among patients with E-BSI of 5.3%, sensitivity, specificity, positive and negative predictive values for the cutoff >5 were: 100%, 83%, 23.5% and 100%, respectively (Table 10). None of the 63 patients with a score ≤5 showed signs or symptoms of IE during the 1-year follow-up.

Figure 5. a)Distribution ofpatients according with the NOVA score: Number of blood cultures (3/3 or the majority if more than 3 = 5 points); Origin of BSI (unknown= 4 points); prior valve disease (2 points); auscultation of heart murmurs (1 point). Dashed line indicate the established cut-off of >5. b) The discriminatory power of NOVA score in the validation cohort was assessed by the receiver operating characteristics (ROC) analysis which showed an area under the curve (AUC) of 0.96. Dashed line representstheidentity line.

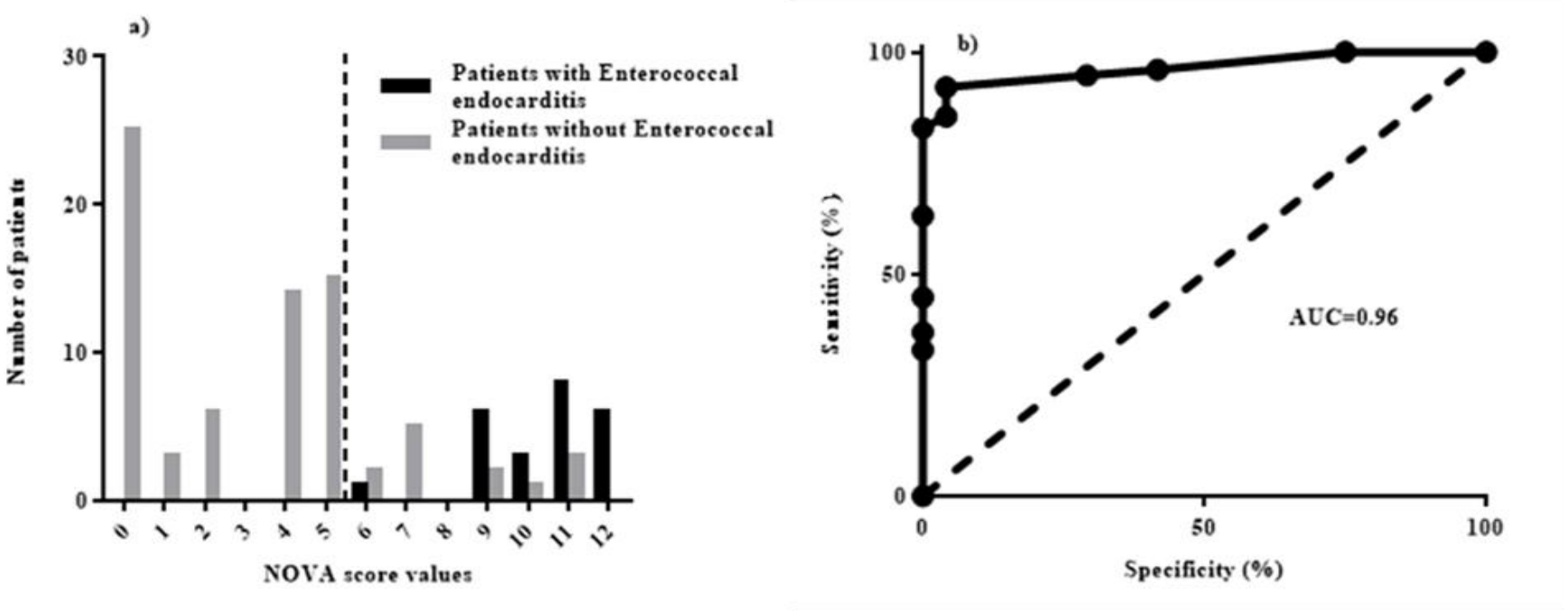


Table 10. Accuracy of NOVA score according with stratified values

Score value	>0	>1	>2	>4	>5	>6	>7	>9	>10	>11
True positive	24	24	24	24	24	23	23	17	14	6
False positive	48	42	34	28	13	11	6	4	3	0
False negative	0	0	0	0	0	1	1	7	10	18
True negative	28	34	42	48	63	65	70	72	73	76
SE (%)	100	100	100	100	100	95.8	95.8	70.8	58.33	25
SP (%)	36.8	44.7	55.2	63.1	82.9	85.5	92.1	94.7	96.5	100
PPV* (%)	7.6	8.6	10.5	12.4	23.5	25.8	38.9	41.2	58.3	100
NPV (%)	100	100	100	100	100	99.7	99.7	98.4	96	96

*Positive predictive value was calculated for a prevalence of infective endocarditis of 5%.

SE sensitivity; SP specificity; PPV positive predictive value; NNV negative predictive value.

Using the model, the percentage of patients with E-BSI who would not require echocardiography (score ≤5 points) was 63% and 78.7% for IE prevalence of 24% and 5%, respectively.

Eleven of the 24 patients with IE developed at least one septic embolic event at level of central nervous system (n=3), spleen (n=3), spinal column (n=3), skin (n=1) and lung (n=1). The crude mortality rate of patient with IE was 25%. In 5 out of the 6 deceased patients the mortality cause was attributed to IE.

III. Systematic performance of new diagnostic imaging techniques in IE patients.

We performed a prospective cohort study (IE patients with PET) involving matched controls in a 1500-bed tertiary hospital attending a population of 700,000 inhabitants. From January 2012 to July 2013, all patients with proven IE underwent PET-CT. The exclusion criteria for PET-CT were hemodynamic instability, pregnancy, surgery during the previous month, clinical intolerance to the test, and known active malignancy. Patients (cases) were matched by affected valve and etiology of definite IE with patients from our cohort who were diagnosed before the PET-CT study was initiated (controls).

Our hospital protocol for detecting septic embolism in patients with IE includes systematic CT of the chest and abdomen or abdominal ultrasound and cranial CT or magnetic nuclear resonance imaging (MRI) if central nervous system symptoms are present. PET-CT was performed simultaneously with these conventional diagnostic techniques.

Patient Preparation for PET-CT. Patients fasted for at least 6 hours before the PET-CT study (i.e. with respect to time of injection of 18F-FDG). If present, hyperglycemia was corrected according to our hospital protocol. PET was not performed if glucose levels were > 160 mg/dl in non-diabetic patients or > 200 mg/dl in diabetic patients at

injection of 18F-FDG. The injection and uptake phase lasted 45 minutes.

According to our hospital protocol, diagnostic craneo-cervico-thoraco-abdominal contrast-enhanced CT scan with intravenous contrast media (OptirayUltraject 300 mg/ml) was performed in all cases, except those with documented allergy, compromised renal function, or concomitant medication (metformin). The intravenous enhanced contrast was administrated when the patient was already laid on the hybrid PET-CT device and this methodology allowed us to avoid the time-lapse between explorations. Oral contrast (barium sulphate 5%, 150 ml, Rovi) was administered in all cases in order to improve the evaluation of the alimentary tract, unless poor tolerance was observed or anticipated. A total of four MBq/Kg of 18F FDG (350-400 MBq) was administered intravenously 60 minutes before imaging with a subsequent rest period of 45 minutes. Afterwards, PET-CT images were acquired with a Siemens Biograph 6-4R truePoint w/true PET-CT device from the vertex to the mid-thigh. Images were reconstructed in axial slices using iterative reconstruction true D.CT images. The attenuation correction was performed with PET / CT fusion in three planes and revised using LEONARDO software (e.soft PET / CT platinum work station. Siemens). All images were evaluated visually and quantitatively by a nuclear physician with PET-CT experience. In doubtful cases, the PET-CT scan was evaluated by at least 2 nuclear

medicine specialists. The CT part of the exam was evaluated independently by an expert radiologist. In the PET-CT scan, the presence or absence of abnormal accumulation of 18F-FDG, especially focal accumulation, was evaluated, as was its size and intensity. Qualitative and semi-quantitative values (maximum standardized uptake value [SUVmax] and mean standardize uptake value [mean SUV]) were recorded for each lesion. Any non-physiological focal uptake superior to that of healthy surrounding tissue (in the case of small lesions approximately <2 cm in size) or uptake superior to reference parenchymal uptake of the mediastinal blood pool or the liver in the case of larger lesions was considered suggestive of pathologically increased metabolic activity. Non-infectious incidental focal hyper-metabolic lesions were classified as neoplasm or inflammation according to radiological, clinical and histological findings, independently of SUV values. Response was assessed by reviewing the images using the same colour scale range, and mean SUV liver uptake values were recorded for both examinations. Images were considered to be comparable in the case of an overlap of x2 SD of liver mean SUV. Both uncorrected and attenuation-corrected images were assessed in order to identify any artefacts caused by contrast agents, metal implants, and/or patient motion.

Definitions and Evaluation Criteria

A true-positive PET-CT result was defined as an abnormal 18F-FDG uptake by any organ or tissue, confirmed as a pathological lesion with clinical, microbiological, and/or standard imaging findings. A false-positive PET-CT result was defined as abnormal 18F-FDG uptake in the absence of clinical and/or microbiological findings, with negative standard imaging results and no relapse during follow-up. Non-infectious incidental PET-CT findings were excluded from the efficacy analysis.

Statistical analysis

The analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA). All tests were 2-sided. A p value <0.05 was considered significant. For the classification of the PET-CT results, we used a discrepant analysis (14), which is recommended for the evaluation of new highly sensitive imaging tests.

Characteristics of the patients

During the study period there were 70 endocarditis episodes, but 23 patients had to be excluded from the study. The reasons for exclusion were early surgery (8 cases), early death (5 cases), discharge (5 cases), admission to the intensive care unit (2 cases), active malignancy (2 cases) and intolerance of the test (1 case). Among excluded patients, IE was caused by *Staphylococcus aureus* (9 cases), *S. epidermidis* (4 cases), unknown (4 cases), *Enterococcus faecalis* (3 cases), *Streptococcus viridans* (2 cases), and *S. pneumoniae* (1 case). Overall mortality during the study period was 29%.

The study population thus included 47 patients from the 70 sequential cases of IE (67.1%).The epidemiological and clinical characteristics of the study patients are shown in table 11. Mean age was 61.3 years (±19 SD) and 30 were male. Infection was caused by Gram-positive microorganisms in 33 cases (70.2%), Gram-negative microorganisms in 4 (8.5%), anaerobes in 5 (10.6%), fungi in 2 (4.2%; *Aspergillus fumigatus* 1 and *Candida parapsilosis* 1), unknown microorganisms in2 (4.2%) and polymicrobial in 1 (2.1%). IE was left-sided in 38 of 47 cases (80.8%), prosthetic valves and/or cardiac devices were affected in 48.9% and 24 (51.4%) of the patients presented a native valve IE.

Table 11.Epidemiological and clinical characteristics of patients with infective endocarditis: assessment of the extension of infection using PET-CT (cases) or conventional imaging methods (controls).

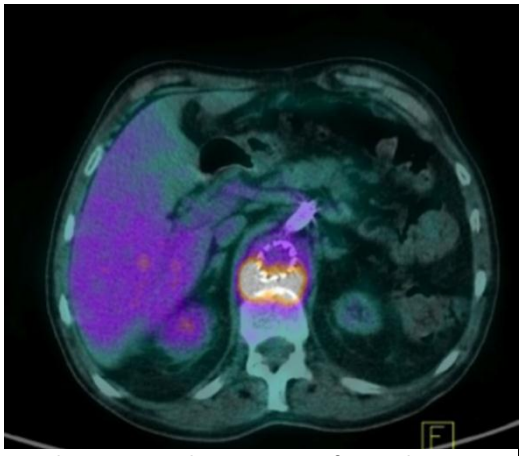
	Cases (n=47)	Controls (n=94)	p
Epidemiology			
Mean age (SD)	61.3 (19)	64.6 (21)	0.34
Sex			0.8
Male	30 (63.8%)	62 (65.9%)	
Female	17 (36.2%)	32 (34.1%)	
Immunosuppression	6 (12.8%)	13 (13.8%)	0.62
HIV infection	0	3	0.21
Transplantation	2	5	0.78
Charlson's comorbidity index	4.09 (± 2.7)	5.05 (± 2.6)	0.13
Previous prosthetic valve	15 (31.9%)	40 (42.5%)	0.22
Aortic	9 (19.1%)	31 (32.9%)	
Mitral	6 (12.7%)	7 (7.4%)	
Pulmonary	0	2 (2.1%)	
Cardiac device	11 (28.5%)	21 (12.85%)	0.88
Pacemaker	8 (17.0%)	18 (19.1%)	
Defibrillator	3 (6.4%)	3 (3.2%)	
Intravascular device	10 (21.2%)	7 (7.4%)	0.01
Central vein catheter	8 (17.0%)	6 (6.4%)	
Other prosthetic material	2 (4.2%)	1(1.0%)	
IE Episode			
Etiology			0.13
Gram-positive	33 (70.2%) *	70 (74.5%)	
Gram-negative	4 (8.5%) **	7 (7.4%)	
Anaerobes	5 (10.6%)***	1 (1%)	
Fungi	2 (4.2%)	4 (4.2%)	
Unknown	2 (4.2%)	10 (10.6%)	
Polymicrobial	1 (2.1%)	2 (2.1%)	
Anatomic distribution of IE			
Prosthetic IE	15(48.6%)	40 (45.7%)	0.22
Aortic	8	20	
Mitral	7	19	
Native IE	24 (51.4%)	50 (54.3%)	0.81
Aortic	12	19	
Mitral	11	25	
Tricuspid	1	6	
Cardiac device	8 (17.0%)	4 (4.2%)	
Outcome			
Treatment-related outcomes			
Days of treatment	43 days (IQR 34-53)	34 days (IQR 17-42)	0.1
Time to effective treatment	3.67 days (+/- 7.8)	13.1 days (+/- 43)	0.15
Persistent BSI	3 (6.4%)	16 (17.0%)	0.08
Valve surgery replacement	30 (63.8%)	39 (41.5%)	0.01
Clinical Outcome			
Hospital stay	39 days (IQR 23-56)	29 days (IQR 17-54)	0.82
Infectious complications	27 (57.4%)	17 (18%)	**0.0001**
Readmission	5 (10.6%)	7 (7.4%)	0.5
Relapse	2 (4.2%)	9 (9.6%)	0.26

IE, infective endocarditis.* *E. faecalis*, 11; *S. aureus*, 5; *S. viridans*, 4; *S. gallolyticus*, 3; *S. epidermidis*, 2; *S. anginosus*, 2; *S. lugdunensis*, 1; *S. gordonii*, 1; *S. pneumoniae*, 1; *group C Streptococcus*, 1; and *A. defectiva*, 1. *group G Streptococcus* 1.**P. aeruginosa*, 3; *H. aphrophillus*, 1. ***A. *actinomycetemcomitans*, 1; *B. thetaiotaomicron*, 1; *C. perfringens*, 1; *L. paracasei*, 1; and *P. acnes*, 1. IQR: interquartile range; BSI: bloodstream infection.

Median length of treatment was 43 days (IQR 34-53 days). Thirty patients (63.8%) underwent valve replacement. The 2 study patients who died were a heart recipient with *A. fumigatus* IE and a massive pulmonary embolism and a patient with *S. epidermidis* IE who presented septic shock. Finally, 1 patient with very extensive *Clostridium perfringens* IE is on the waiting list for heart transplantation.

PET-CT Results

PET/CT showed at least 1 lesion in 35 patients (74.5%). The 46 affected sites were as follows: lung, 10 (21.7%); bone, 7 (15.2%); sigmoid, rectum, and anus, 8 (17.4%); soft tissue 4 (8.7%) spleen, 3 (6.5%); brain and prosthetic valve, 4 each (17.4%); and extra-cardiac Fontan tube, aortic homograft, pulmonary valve graft, intravascular prosthetic material, right atrium and diaphragm (1 each).The classification of PET-CT results according to our definitions is shown in table 12. Five patients (10.6%) had a non-infectious PET-CT finding (lung cancer, colonic adenocarcinoma, lymphocytic interstitial pneumonia, diverticulosis and solitary lung nodule).



A male patient with an aortic graft prosthetic material infectio

None of thefindings were identified by the conventional radiological extension study. These incidental findings were excluded from the efficacy analysis.

A femalepatientwith a fontancardiactube and Group C Streptococcus IE

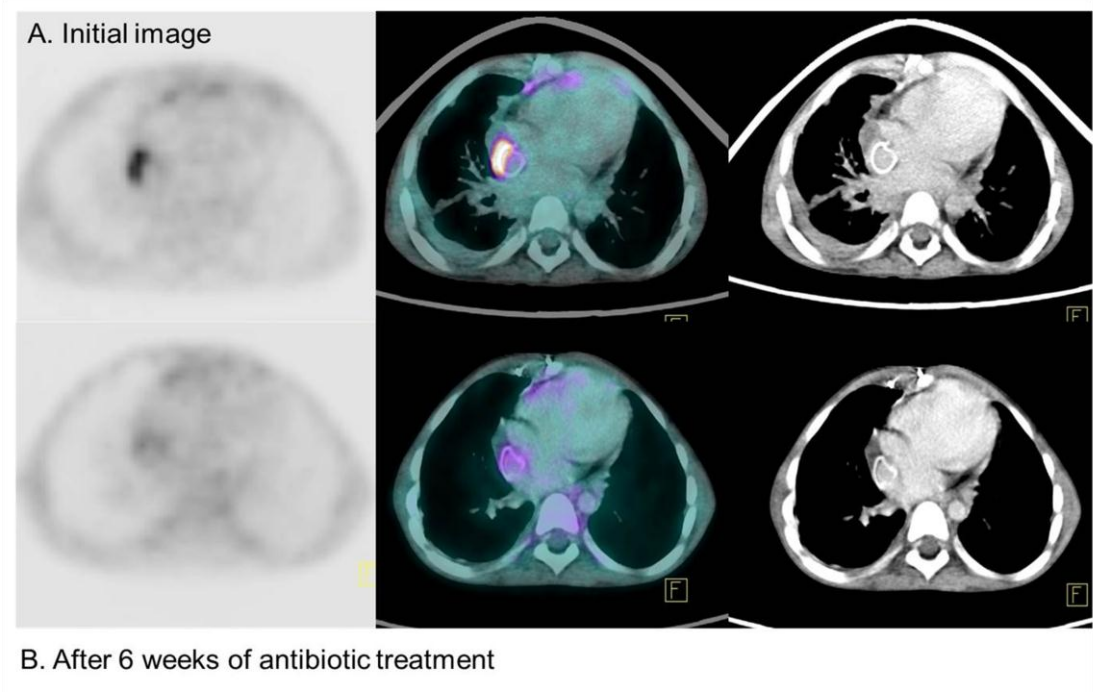


Table 12: Description of the 47 patients with IE in whom 18 FDG PET/CT was performed.

Age/ Sex	Microorganism	PET-CT uptake sites	Signs or symptoms	Initial imaging technique	Classification	Treatment implication
73/F	*P. aeruginosa*	Spondylodiscitis D12. Perm-cath. Pulmonary septic embolisms	No	Negative	Very significant True positive*	Therapy prolonged. Perm-cath withdrawn
68/M	*C. perfringens*	Rectum and anus abscess	No	Negative	Very significant True positive *	Therapy prolonged. Origin of the IE identified
68/M	*S. viridans*	Spondylodiscitis, Rectum and sigmoid	Yes	Negative	Very significant True positive*	Therapy prolonged and colonoscopy performed to rule out malignancy
75/M	*E. faecalis*	Spondylodiscitis L1-L2	Yes	Negative	Very significant True positive*	Therapy prolonged
5/F	*Group C Streptococccus*	Extracardiacfontan tube	No	Negative	Very significant True positive*	Therapy prolonged
81/M	*A. defectiva*	Spondylodiscitis L1-L2, L4-L5	Yes	Negative	Very significant True positive*	Therapy prolonged
83/M	*E. faecalis*	Spondylodiscitis L1 L2 L3	Yes	Negative	Very significant True positive*	Therapy prolonged
22/M	*L. paracasei*	Spleen embolism	No	Negative	Very significant True positive*	Therapy prolonged
94/M	*C. parapsilosis*	Pulmonary septic embolisms	No	Negative	Very significant True positive*	Therapy prolonged
80/M	*Unknown*	Aortic graft prosthetic material	No	Negative	Very significant True positive*	Therapy prolonged
50/F	*S. aureus*	Pulmonary valve after tricuspid valve replacement	No	Negative	Very significant True positive*	Closer TEE follow-up
79/M	*S. epidermidis*	Soft tissue around pacemaker	No	Negative	Very significant True positive*	Relapsing BSI and septic shock
2/F	*P. aeruginosa*	Pulmonary valve graft, and pulmonary septic embolisms	Yes	Negative	Very significant True positive*	Surgical removal of valve graft and therapy prolonged
35/M	*S. aureus*	Brain and pulmonary embolisms	Yes	Negative	Very significant True positive*	Therapy prolonged
68/M	*S. aureus*	Aortic homograft	No	Negative	Very significant True positive*	Aortic valve replacement
33/M	*S. lugdunensis*	Pulmonary embolisms	No	Positive	True positive	Therapy prolonged

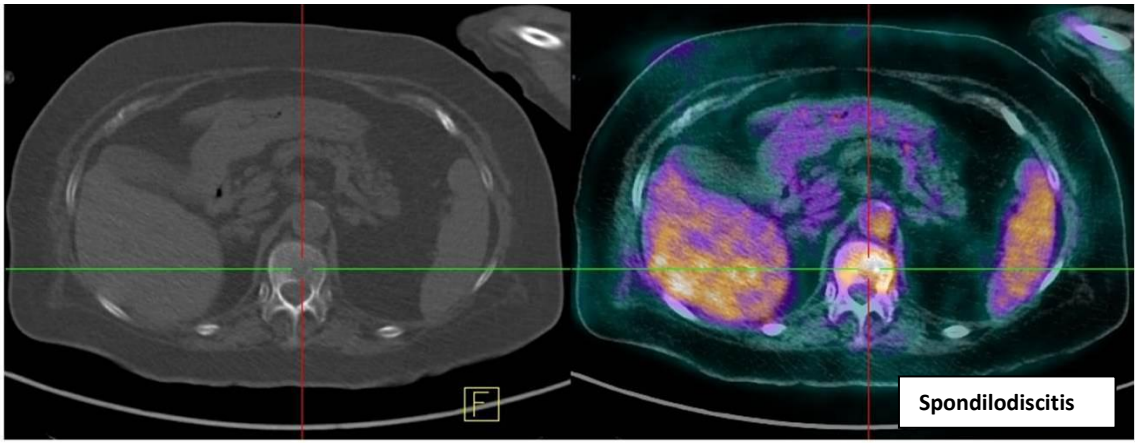
50/M	*B. thetaiotaomicron*	Spleen embolism	No	Positive	True positive	Therapy prolonged
54/F	*A. fumigatus*	Pulmonary embolism	Yes	Positive	True positive	Therapy prolonged
78/F	*S. gallolyticus*	Pleural effusion	Yes	Positive	True positive	Completed IE treatment
72/M	*H. aphrophillus*	Brain embolism	Yes	Positive	True positive	Therapy prolonged
70/M	*E. faecalis*	Spleen abscess	No	Positive	True positive	Therapy prolonged
87/F	*S. viridans*	Aortic prosthetic valve	No	Positive	True positive	Completed IE treatment
67/M	*E. faecalis*	Rectal wall thickness	No	Positive	True positive	Therapy prolonged
70/M	*A. actinomycetemcomitans*	Brain embolism	Yes	Positive	True positive	Found origin of IE Therapy prolonged
80/F	*E. faecalis*	Rectal wall thickness	Yes	Positive	True positive	Completed IE treatment
33/F	*Group G streptococcus*	Right atrium emboli	Yes	Positive	True positive	Completed IE treatment
83/M	*E. faecalis*	Pacemaker	No	Positive	True positive	Completed IE treatment
55/M	*S. gordonii*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
76/V	*E. faecalis*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
83/F	*P. aeruginosa*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
39/F	*S. aureus*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
73/F	*S. viridans*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
53/M	*S. viridans*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
36/M	*S. gallolyticus*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
51/M	*E. faecalis*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
30/M	*S. anginosus*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
62/F	*S. epidermidis*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
83/M	*S. aureus*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
45/M	*E. faecalis*	Non-significant uptake	No	Negative	True negative	Completed IE treatment
60/M	*S. gallolyticus*	Left radio	No	Negative	False positive	No modifications
59/F	*E. faecalis*	Diaphragm	No	Negative	False positive	Closer follow-up
76/F	*S. aureus*	Soft tissue	No	Negative	False positive	Closer follow-up

84/F	*S. viridans*	Sigmoid and colon	No	Negative	Incidental finding	Colonic adenocarcinoma diagnosed
65/M	*P. acnes*	Left pectoral muscle and lung nodule	No	Negative	Incidental finding	Lung adenocarcinoma diagnosed
70/F	*S. pneumoniae*	Multiple lung lesions	No	Negative	Incidental finding	Lymphocytic interstitial pneumonia
68/M	*E. faecalis*	Solitary lung nodule	No	Negative	Incidental finding	Lung cancer study
64/M	*S. mitis*	Diverticulosis	No	Negative	Incidental finding	Colonic study

IE, infectious endocarditis; BSI, bloodstream infection; TEE, transesophageal echocardiography.

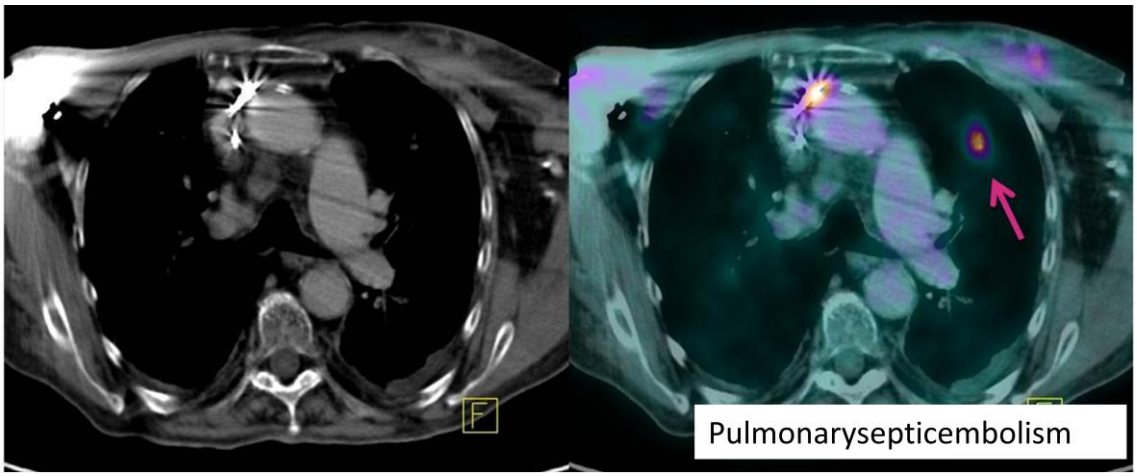
* True positives where 18 FDG PET/CT was the first diagnostic tool that identify the complication.

The validity values for efficacy of PET-CT for the diagnosis of infectious embolism were as follows: sensitivity, 100%; specificity, 80%; positive predictive value, 90%; and negative predictive value, 100%. Overall, 27 patients (57.4%) were classified as true positives. Only 12 of the 27 true-positive results (44.4%) were initially identified in the conventional extension study, as follows: lung, 3; spleen, 2; brain, 2; rectal wall, 3; and aortic prosthetic valve, and pleura (1 each).



In the remaining 15 true-positive cases (55.5%), PET-CT was the only initial positive imaging technique: 5 cases of spondylodiscitis (SUVmax, 5.39), 3 cases of intra/endovascular prosthetic material infection (SUVmax, 7.39), 3 cases of septic pulmonary embolism (SUVmax, 3.75), 2 sigmoid, rectum, and anus lesions (SUVmax, 7.53), 1 case of septic spleen embolism (SUVmax, 5.60), 1 case of brain embolism (SUVmax 7.8) 1 pulmonary valve graft (SUVmax 4.33) and 1 soft tissue around the pacemaker (SUVmax, 3.74)(Table 12).These findings resulted in prolongation of antibiotic treatment for a mean of 52 days (SD 49 days).

Twelve patients (25.5%) were classified as true negatives, since both PET-CT and conventional imaging techniques excluded the presence of complications (Table 2). Median length of therapy in these patients was 34.5 days (IQR, 12.25-46.5), and all patients remained asymptomatic during follow-up.



Comparison of Cases and Controls

In order to assess the clinical impact of PET-CT in patients with IE, we compared the study cases with a historic control cohort (1:2) from our database matched for etiology and site of IE. The epidemiological and clinical characteristics of cases and controls were similar (Table 11). Systematic use of PET-CT led to a statistically significant increase in the diagnosis of infectious complications (57.4 % vs. 18.0%; p = 0.0001). Although the difference did not reach statistical significance due to the low number of cases, PET-CT was associated with a 2-fold reduction in the number of relapses (4.2% vs.9.6 % p = 0.25).

Hospital stay remained stable, mainly owing to the support of the outpatient parental antibiotic therapy program.

There were 3 false-positive PET results (6.4%) in patients with abnormal uptake on their initial PET-CT that was not subsequently confirmed (Table 2). The sites included left radius (SUVmax, 3.80), diaphragm (SUVmax, 7.56), and soft tissue (SUVmax, 2.15) (Table 12). None of them presented associated clinical signs or symptoms, follow-up by conventional imaging was negative, treatment was not modified (median of 45 days), and all patients remained asymptomatic during follow-up. No false-negative results were detected.

DISCUSSION

I.

Our very large series, which was collected from different institutions in a single country over a short period of time, shows epidemiologic changes in IE. Nowadays, IE is a disease of the elderly, with multiple morbidity conditions; frequently nosocomial (28.1%) and had very high mortality both during admission and during the one-year follow-up.

Incidence rates of IE have been collected over long periods of time, and data based on population studies are scarce. Incidence rates range from 3-10 cases/100,000 habitants [21, 23, 27, 51, 76] and our figure of 3.5 IE cases/100,000 habitants in a whole country study is concordant with that.

The underlying conditions of patients with IE have also drastically change [20, 21, 51], and most of our cases presented with severe comorbid conditions. This population of fragile patients are frequently exposed to health care–related and nosocomial complications. In our series, 28.1% of the episodes were classified as nosocomial; this percentage is similar to that reported by Fernandez-Hidalgo et al [77] in a series from Spain (28.4%).

As well as the epidemiology, clinical presentation of the disease has changed, and the signs that were once typical of IE (splinter hemorrhages [2.3%], Janeway lesions [2.4%], and Osler nodes [1.9%]) are now uncommon. One possible explanation is that IE patients are

now diagnosed earlier (86% were admitted ≤ 1 month of the initial signs of illness), thus reducing the incidence of immunological manifestations [18].

On the other hand, we commonly observed complications such as respiratory manifestations (41%), kidney failure (39%), neurological events (19.7%), osteoarticular symptoms (11.5%), and ocular manifestations (6.3%). The rate of embolic events in our series was 29%, and although our results are similar to the ones reported by others [18, 78], we believe that this figure could be underestimated, since the extension study depends on the institutional protocol and the technology available in each center. The introduction of newer diagnostic imaging tools such as PET-CT as part of the diagnostic algorithm in patients with IE, as suggested by Saby *et al* [68], should prove to be of great interest in this field.

A shift in the type of patient with IE has been observed: one major change in our series was the very low proportion of IE now occurring in IVDU. In Spain, this is due, without question to the very large programs to control IVDU´s and particularly the methadone maintenance programs [79-82]. Although historically native valve IE in IVDU represented an important number of affected patients [83], in our series, this population accounted for the smallest group whilst the number of patients with prosthetic valve and device IE (37.3%), on the other hand, has risen [21, 27].

Microbiological diagnostic tools have changed and improved over recent decades and, although etiology was confirmed by blood culture in most cases (85.3%), there are still cases in which the etiology is unknown (9.1%). Molecular techniques enabled us to establish the etiology in 20.7% of the negative blood culture episodes that would otherwise have been considered IE of unknown etiology. However, even though molecular methods have been used to diagnose IE are long time well know [84], this diagnostic approach is still not routinely available in all diagnostic laboratories.

As for mortality, IE is a severe disease with a poor outcome. In our series, in-hospital mortality was 28.9% and one-year mortality was 11.2%. It seems that infective endocarditis mortality despite the introduction of broad-spectrum antibiotics and new diagnostic tools, the mortality of IE has remained close to 25% since the 1970s [20]. The only intervention that has shown a major impact on mortality was surgery, since it was independently associated with a decreased risk of one-year mortality (OR, 0.44); our results agree with those of a recent analysis of published studies [85] that shows a significant correlation between the rate of early surgery and mortality.

The most common microorganisms in our series were staphylococci, streptococci, and enterococci. Thus, a major change in the microbiology of IE is that *Enterococcus* spp has emerged as the third most important group of pathogens and is now responsible for 12.7% of IE cases. Since

enterococci have shown the ability to develop antibiotic resistance[49],the frequency of E-BSI is growing, and a significant percentage of cases are associated with IE, we decided to evaluate a novel approach to this increasingly frequent problem [86] and specially try to identify factors that enable the early selection of patients who are at risk for enterococcal IE.

II

In our institution enterococcal IE is present in 4.3% of all patients with E-BSI, 16.7% of patients undergoing echocardiography and in 35.5% of those who undergo TEE. A simple, bedside predictive score allowed us to identify a subgroup of patients with E-BSI in whom the risk of enterococcal IE is very low and who therefore would not require systematic TEE. Our results confirm that, as indicated in the American guidelines, TEE should be the test of choice when the indication is to detect IE, especially if the pre-test probability is high, such as in patients with staphylococcal bacteremia, fungemia, prosthetic heart valve, or intracardiac device [87]. Although systematic performance of TEE is not recommended in patients with enterococcal bacteremia in current guidelines, our relatively small percentage of patients with enterococcal bacteremia who underwent TEE (25.6%), reflects the real daily practice and so far, is the only figure in the literature. In our opinion, TEE should also be performed in patients with enterococcal IE and a NOVA score >4 points.

In many institutions, Enterococcus species is the third most common cause of BSI. The main origins are the gastrointestinal tract and catheter-related infections[26]. The need to rule out IE in patients with staphylococcal bacteremia remains open to debate [88], although some authors suggest that it may be unnecessary in 26-28% of patients fulfilling specific criteria of uncomplicated BSI [89, 90]. The indication for echocardiography is even less clear in episodes of bacteremia caused by Streptococcus, Candida, and Enterococcus species [62].

Current guidelines for the diagnosis of IE [62] include echocardiography as a key test for the diagnosis and management of patients with IE; however, whether TTE or TEE should be performed first depends on the interpretation of a series of complex clinical, microbiological, and radiological findings. Although the detection rate for TTE is approximately 50%[60], the efficiency of the technique is affected by factors such as image quality, presence of previous valve disease or prosthetic material, skill of the examiner, and pre-test probability of endocarditis. It has been proposed that for patients with a high probability of endocarditis, performing TEE provides the highest quality-adjusted survival [88]. Our study suggests that most patients with E-BSI (those with a score ≥4 points) should undergo TEE (16.7% positive).

We also show that TTE misses >70% of episodes of IE and that those patients should undergo TEE. Our risk prediction score (NOVA)

provides physicians with an easy-to-use system that could rapidly determine which patients with E-BSI may require further studies to detect IE, merely by examining the number of positive blood cultures, the origin of the bacteremia, previous history of valve disease, and auscultation of a heart murmur.

Our study is subject to a series of limitations. First, as it was performed in a single center, the sample size was not as large as it might have been. Nevertheless, it is the largest sample reported to date and was collected over a long period. Second, since patients with bacteremia were previously evaluated by an infectious diseases specialist, selection bias should be taken into consideration. Interestingly, despite the intervention of the infectious diseases department, the rate of compliance with echocardiography recommendations remains low (34.4%), and a significant number of patients (1127) with enterococcal bacteremia did not undergo TEE, thus limiting the ability of the study to estimate the real prevalence of IE. However, a further analysis of this population showed that only a very small proportion of patients (1.7%) could have had IE and that even without TEE, they were treated for at least 4 weeks. Finally, ours is a case-control study and the results should be validated in a second cohort and/or prospective study.

Overall, the NOVA score is particularly useful for identifying a subgroup of patients with enterococcal bacteremia who may not need to undergo TEE (sensitivity 100%) because of an extremely low risk of endocarditis.

We do not aim to put forward a hypothesis on the treatment of bacteremia or endocarditis, but believe that treatment should be established according to guidelines, predisposing conditions, or clinical presentation (e.g. stroke and embolic phenomena) independently of the NOVA score.

The first validation of the NOVA score in an external cohort of patients with E-BSI shown that this model is very useful for discriminating patients at low risk for IE and therefore, it could be used to select patients in whom an echocardiography for ruling out IE could be safely obviated. However, a prospective study in which all the patients with E-BSI will be uniformly studied for IE, using not only echocardiography, is needed to definitely validate the NOVA score.

III

The outcome of IE is closely associated with the extent of systemic embolization and extra-cardiac infection; most relapses are due to insufficient duration of original treatment or a persistent focus of infection [62]. However, a diagnosis of peripheral septic embolism is often challenging. Current guidelines [62] agree that embolic events can be totally silent in 20% of cases, especially those affecting the spleen or cerebral blood flow, and can only be detected using imaging techniques (abdominal and cerebral CT scan). To date, no clear consensus has been reached on which imaging technique should be performed or

whether imaging should be performed systematically or only in symptomatic patients. In many cases, the extension study requires multiple tests that are not only time-consuming, but also expensive and troublesome for the patient.

The introduction of PET-CT to investigate tumor extension in oncology revolutionized the practice of medicine [63]; the assessment of hypermetabolic lesions in the field of infectious diseases is more recent [61]. In 2010, Vos *et al* [64] used PET-CT technology to assess distant infectious lesions in 115 neutropenic patients with Gram-positive non-endocarditic bloodstream infections. Metastatic infectious foci were detected in 35% of patients; in half, the diagnosis was not previously suspected. Subsequently, PET-CT has been evaluated to investigate high-uptake lesions in the heart or in intracadiac devices [68, 91, 92].

Studies evaluating the role of PET-CT in ruling out extracardiac involvement in patients with endocarditis are mainly case reports [67]. Van Riet *et al,* studied 25 patients with IE and found infectious septic embolisms in about 44% of patients [66]; however, the study did not have a control group, nor did it attempt to evaluate the clinical impact of these findings. Our study showed that 57.4% of patients were eventually diagnosed with an infectious complication and that more than half (60%) were asymptomatic. PET-CT makes it possible to detect infectious embolism throughout the body in a single easily performed test (<2 h) that is comfortable for the patient and provides the clinician

with whole-body data. Although, it was not the purpose of this study to evaluate de heart valve lesions with PET-CT, but to evaluate extra-cardiac involvement, PET-CT detected 7 of 8 cases of IE related to extra-cardiac prosthetic material: 3 defibrillators (2 with septic pulmonary embolisms and 1 with subcutaneous abscess), 1 aortic graft infection, 1 extra-cardiac Fontan tube infection, 1 aortic homograft and 1 central vein catheter (pulmonary septic embolisms). These findings agree with those of Sarrazin *et al* [92], who also found that PET-CT was a useful tool for the diagnosis of cardiovascular implantable electronic device infections and assessment of their extension. Gated studies of the heart, a previous diet preparation of the patient and a larger acquisition time would definitely increase the detection of heart valve lesions, but more information is needed on this aspect.

The systematic performance of PET-CT made it possible to detect other diseases, such as cancer, that could be involved in the pathogenesis of endocarditis. Although the pathophysiological relationship between endocarditis and neoplasm remains unclear, the simultaneous finding of both entities is not rare [93]. Thomsen *et al,* [94] have recently proposed that endocarditis is a substantial clinical marker for the presence of occult cancer with a standardized incidence rate (SIR) of 1.61 (CI: 1-5-1.71). In this series, cancer risk in endocarditis patients was highly elevated during the first 3 months of follow up (SIR= 8.03; 95% CI, 6.92-9.26). In our series, PET-CT enabled early detection of 2

tumors (1 lung and 1 colon) in patients with *Propionibacterium acnes* and *S. anginosus* IE.

The conventional extension study included 76 imaging techniques (1.61 tests per patient) that included 27 CT´s, 25 echography´s, 13 x-rays, 5 MRI´s, 5 gammagraphies and 1 angio-CT. PET-CT is clearly more expensive than conventional CT or MRI (€658/patient vs. €326.42/patient), although it has considerably improved the diagnosis of infectious complications. In 15 of 27 cases (55.5%), PET-CT was the only initial positive imaging technique that revealed an infectious complication. Based on data from our health authorities [95], the mean extra-cost of a major complication of a systemic infection is €20,241; therefore, early diagnosis of infectious complications with PET-CT is cost-effective. Vos *et al,* [64] evaluated the cost-effectiveness of routine PET-CT in 115 high-risk patients with Gram-positive bacteremia and found that the cost-effectiveness ratio was $72,487 per prevented death.

Our study is subject to a series of limitations. First, it was performed in a single center, thus reducing the number of patients who could be included. Second, early PET-CT could only be performed in 66% of patients with infective endocarditis, mainly because of emergency surgery and hemodynamic instability, which probably excluded patients with the most severe complications. Because of inflammatory changes and hemodynamic instability after cardiac surgery, we decided not to

perform the test immediately in patients who had recently undergone surgery. This problem could be resolved as experience with the technique increases. Furthermore, our institution only has 1 PET-CT device, and cancer patients have preference on the waiting list. Therefore, scheduling was problematic in some cases; however, given increasing evidence of the usefulness of PET-CT in patients with infectious diseases, we expect this situation to change. Third, PET-CT is a highly sensitive test for localizing abnormalities, since results are a measure of inflammatory cell activity [96]; therefore, the results could increase the risk of false-positive findings. To minimize this effect, we performed a discrepant analysis and compared PET-CT results with clinical and microbiological data and the results of conventional imaging techniques. The most problematic discrepant results were those for the 3 patients in whom PET-CT findings could not be confirmed by clinical, microbiological, or radiological findings during the course of their disease. For the sake of this study we considered these results to be false positives. Fourth: to assess the impact of PET-CT on mortality or relapse in patients with infective endocarditis a prospective randomized study, with a larger number of subjects should be performed.

Overall, our study shows that PET-CT is a more effective way of assessing the extension of infection in patients with IE.

CONCLUSIONS

I. IE remains an infrequent but severe disease that commonly presents in older patients with multiple underlying conditions and is frequently health care–related.

II. Multidisciplinary groups are essential to optimize the management and outcome of IE; so far, the only intervention that has shown a major impact on one year mortality was surgery.

III. The prevalence of enterococcal IE depends on whether the sample comprised all cases among those with E-BSI (4.3%), only patients undergoing echocardiography (16.7%), or only patients undergoing TEE (35.5%).

IV. Depending on the local prevalence of endocarditis, application of the NOVA bedside prediction score could safely obviate echocardiography in 14-27% of patients with enterococcal bacteremia.

V. PET-CT is an effective way of accomplishing the extension study in a single test in patients with IE. It is easily performed (<2 h) and comfortable for the patient and provides the clinician with whole-body data.

VI. PET-CT enables significantly more infectious complications to be diagnosed (18.0% vs. 57.4%; p = 0.0001) and its use procured a trend toward a reduced number of relapses (9.6 % vs. 4.2% p = 0.25) in patients with IE.

REFERENCES

1. Contrepois, A., Towards a history of infective endocarditis. Medical History, 1996. 40(1): p. 25-54.
2. Fleck, L. and T.J. Trenn, Genesis and Development of a Scientific Fact. 1981: University of Chicago Press.
3. Cunningham, A.a.W., Perry, The laboratory revolution in medicine, ed. C.U. Press. 1992. 349.
4. Millar, B.C. and J.E. Moore, Emerging issues in infective endocarditis. (1080-6040 (Print)).
5. Maulitz, R.C., et al., Grand rounds: one hundred years of internal medicine. 1988: University of Pennsylvania Press.
6. Longcope, W., Sir William Osler and bacterial endocarditis. 1949: Hopkins Hosp.
7. Bourne, G., Lord Horder. (0007-0769 (Print)).
8. Favour Cb Fau - Janeway, C.A., C.A. Janeway, and et al., Progress in the treatment of subacute bacterial endocarditis. (0028-4793 (Print)).
9. Wallace Ag Fau - Young, W.G., Jr., S. Young Wg Jr Fau - Osterhout, and S. Osterhout, TREATMENT OF ACUTE BACTERIAL ENDOCARDITIS BY VALVE EXCISION AND REPLACEMENT. (0009-7322 (Print)).

10. Durack, D.T., A.S. Lukes, and D.K. Bright, New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. Am J Med, 1994. 96(3): p. 200-9.
11. Li, J.S., et al., Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis, 2000. 30(4): p. 633-8.
12. Fauci AS, K.D., Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J, ed. Harrison's Principles of Internal Medicine. 17 ed. 2008, McGraw-Hill: New York. 2607.
13. Keynan, Y. and E. Rubinstein, Pathophysiology of infective endocarditis. Curr Infect Dis Rep, 2013. 15(4): p. 342-6.
14. Jung, C.J., et al., Platelets enhance biofilm formation and resistance of endocarditis-inducing streptococci on the injured heart valve. J Infect Dis, 2012. 205(7): p. 1066-75.
15. Sande, M.A. and W.M. Scheld, Bacterial adherence in endocarditis: interaction of bacterial dextran, platelets, fibrin, and antibody. Scand J Infect Dis Suppl, 1980. Suppl 24: p. 100-5.
16. Mandell, G.L., Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, C.L. ELSEVIER, Editor. 2010. p. 4320.

17. Baddour, L.M., Infective Endocarditis: Diagnosis, Antimicrobial Therapy, and Management of Complications: A Statement for Healthcare Professionals From the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: Endorsed by the Infectious Diseases Society of America. Circulation, 2005. 111(23): p. e394-e434.
18. Murdoch, D.R., et al., Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. Arch Intern Med, 2009. 169(5): p. 463-73.
19. Bor, D.H., et al., Infective endocarditis in the U.S., 1998-2009: a nationwide study. PLoS One, 2013. 8(3): p. e60033.
20. Slipczuk, L., et al., Infective endocarditis epidemiology over five decades: a systematic review. PLoS One, 2013. 8(12): p. e82665.
21. Bouza, E., et al., Infective endocarditis--a prospective study at the end of the twentieth century: new predisposing conditions, new etiologic agents, and still a high mortality. Medicine (Baltimore), 2001. 80(5): p. 298-307.

22. Alagna, L., et al., Repeat endocarditis: analysis of risk factors based on the International Collaboration on Endocarditis - Prospective Cohort Study. Clin Microbiol Infect, 2013.
23. Hoen, B., et al., Changing profile of infective endocarditis: results of a 1-year survey in France. JAMA, 2002. 288(1): p. 75-81.
24. Rodriguez-Creixems, M., et al., Bloodstream infections: evolution and trends in the microbiology workload, incidence, and etiology, 1985-2006. Medicine (Baltimore), 2008. 87(4): p. 234-49.
25. Alonso-Valle, H., et al., The changing face of prosthetic valve endocarditis at a tertiary-care hospital: 1986-2005. Rev Esp Cardiol, 2010. 63(1): p. 28-35.
26. Reigadas, E., et al., Catheter-related bloodstream infection caused by Enterococcus spp. Clin Microbiol Infect, 2013. 19(5): p. 457-61.
27. Tornos, P., et al., Infective endocarditis in Europe: lessons from the Euro heart survey. Heart, 2005. 91(5): p. 571-5.
28. Fernandez-Guerrero, M.L., et al., Nosocomial enterococcal endocarditis: a serious hazard for hospitalized patients with enterococcal bacteraemia. J Intern Med, 2002. 252(6): p. 510-5.
29. Jorgensen James, C.K., Funke Guido, Pfalle Michael., Manual of Clinical Microbiology. 11 ed. Vol. 1. 2015, Washington Dc. 2571.
30. Murray, P.R., Manual of Clinical Microbiology. 2V. 2003: ASM Press.

31. Goldman, E. and L.H. Green, Practical Handbook of Microbiology, Second Edition. 2008: CRC Press.
32. Gilmore, M.S., The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. 2002: ASM Press.
33. Jorgensen, J.H. and M.A. Pfaller, Manual of Clinical Microbiology. 2015: ASM Press.
34. Franz, C.M., et al., Enterococci in foods--a conundrum for food safety. Int J Food Microbiol, 2003. 88(2-3): p. 105-22.
35. Franz, C.M., et al., Enterococci as probiotics and their implications in food safety. Int J Food Microbiol, 2011. 151(2): p. 125-40.
36. Wijaya, A., et al., Cloning of the bile salt hydrolase (bsh) gene from Enterococcus faecium FAIR-E 345 and chromosomal location of bsh genes in food enterococci. J Food Prot, 2004. 67(12): p. 2772-8.
37. Ben Omar, N., et al., Functional and safety aspects of Enterococci isolated from different Spanish foods. Syst Appl Microbiol, 2004. 27(1): p. 118-30.
38. Franz, C.M., et al., Bile salt hydrolase activity of Enterococci isolated from food: screening and quantitative determination. J Food Prot, 2001. 64(5): p. 725-9.

39. Facklam, R.R. and M.D. Collins, Identification of Enterococcus species isolated from human infections by a conventional test scheme. J Clin Microbiol, 1989. 27(4): p. 731-4.

40. Gordon, S., et al., Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States. Enterococcal Study Group. J Clin Microbiol, 1992. 30(9): p. 2373-8.

41. Forrest, G.N., et al., Single center experience of a vancomycin resistant enterococcal endocarditis cohort. J Infect, 2011. 63(6): p. 420-8.

42. Malone, D.A., et al., Enterococcal bacteremia in two large community teaching hospitals. Am J Med, 1986. 81(4): p. 601-6.

43. Sava, I.G., E. Heikens, and J. Huebner, Pathogenesis and immunity in enterococcal infections. Clin Microbiol Infect, 2010. 16(6): p. 533-40.

44. Garbe, J., et al., EndoE from Enterococcus faecalis hydrolyzes the glycans of the biofilm inhibiting protein lactoferrin and mediates growth. PLoS One, 2014. 9(3): p. e91035.

45. Matos, R.C., et al., Enterococcus faecalis prophage dynamics and contributions to pathogenic traits. PLoS Genet, 2013. 9(6): p. e1003539.

46. Baldassarri, L., et al., Pathogenesis of implant infections by enterococci. Int J Artif Organs, 2005. 28(11): p. 1101-9.

47. Anderson, D.J., et al., Enterococcal prosthetic valve infective endocarditis: report of 45 episodes from the International Collaboration on Endocarditis-merged database. Eur J Clin Microbiol Infect Dis, 2005. 24(10): p. 665-70.

48. Barie, P.S., et al., Pathogenicity of the enterococcus in surgical infections. Ann Surg, 1990. 212(2): p. 155-9.

49. Arias, C.A. and B.E. Murray, The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol. 10(4): p. 266-78.

50. Hidron, A.I., et al., NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol, 2008. 29(11): p. 996-1011.

51. Fernandez-Hidalgo, N. and P. Tornos Mas, Epidemiology of Infective Endocarditis in Spain in the Last 20 Years. Rev Esp Cardiol, 2013. 66(9): p. 728-733.

52. Shlaes, D.M., J. Levy, and E. Wolinsky, Enterococcal bacteremia without endocarditis. Arch Intern Med, 1981. 141(5): p. 578-81.

53. Patterson, J.E., et al., An analysis of 110 serious enterococcal infections. Epidemiology, antibiotic susceptibility, and outcome. Medicine (Baltimore), 1995. 74(4): p. 191-200.

54. Garrison, R.N., et al., Enterococcal bacteremia: clinical implications and determinants of death. Ann Surg, 1982. 196(1): p. 43-7.

55. Anderson, D.J., et al., Risk factors for infective endocarditis in patients with enterococcal bacteremia: a case-control study. Infection, 2004. 32(2): p. 72-7.

56. Fernandez-Cruz, A., et al., The value of combining blood culture and SeptiFast data for predicting complicated bloodstream infections caused by Gram-positive bacteria or Candida species. J Clin Microbiol, 2013. 51(4): p. 1130-6.

57. McDonald, J.R., et al., Enterococcal endocarditis: 107 cases from the international collaboration on endocarditis merged database. Am J Med, 2005. 118(7): p. 759-66.

58. Wilson, W.R., et al., Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. Ann Intern Med, 1984. 100(6): p. 816-23.

59. Rice, L.B., et al., Enterococcal endocarditis: a comparison of prosthetic and native valve disease. Rev Infect Dis, 1991. 13(1): p. 1-7.

60. Evangelista, A. and M.T. Gonzalez-Alujas, Echocardiography in infective endocarditis. Heart, 2004. 90(6): p. 614-7.

61. Glaudemans, A. and A. Signore, FDG-PET/CT in infections: the imaging method of choice? Eur J Nucl Med Mol Imaging, 2010. 37(10): p. 1986-91.

62. Habib, G., et al., Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J, 2009. 30(19): p. 2369-413.

63. Kubota, R., et al., Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. J Nucl Med, 1992. 33(11): p. 1972-80.

64. Vos, F.J., et al., Cost-Effectiveness of Routine 18F-FDG PET/CT in High-Risk Patients with Gram-Positive Bacteremia. Journal of Nuclear Medicine, 2011. 52(11): p. 1673-1678.

65. Vos, F.J., et al., 18F-FDG PET/CT for Detection of Metastatic Infection in Gram-Positive Bacteremia. Journal of Nuclear Medicine, 2010. 51(8): p. 1234-1240.

66. Van Riet, J., et al., 18F-FDG PET/CT for early detection of embolism and metastatic infection in patients with infective endocarditis. Eur J Nucl Med Mol Imaging, 2010. 37(6): p. 8.

67. Millar, B.C., et al., 18FDG-positron emission tomography (PET) has a role to play in the diagnosis and therapy of infective endocarditis and cardiac device infection. International journal of cardiology, 2013.

68. Saby, L., et al., Positron Emission Tomography/Computed Tomography for Diagnosis of Prosthetic Valve EndocarditisIncreased Valvular 18F-Fluorodeoxyglucose Uptake as a Novel Major Criterion. Journal of the American College of Cardiology, 2013. 61(23): p. 2374-2382.

69. Cockerill, F.R., 3rd, et al., Optimal testing parameters for blood cultures. Clin Infect Dis, 2004. 38(12): p. 1724-30.

70. Roques, F., et al., Risk factors and outcome in European cardiac surgery: analysis of the EuroSCORE multinational database of 19030 patients. Eur J Cardiothorac Surg, 1999. 15(6): p. 816-22; discussion 822-3.

71. Nashef, S.A., et al., Validation of European System for Cardiac Operative Risk Evaluation (EuroSCORE) in North American cardiac surgery. Eur J Cardiothorac Surg, 2002. 22(1): p. 101-5.

72. Charlson, M.E., et al., A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis, 1987. 40(5): p. 373-83.

73. Adams, H.P., Jr., et al., Guidelines for the management of patients with acute ischemic stroke. A statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke, 1994. 25(9): p. 1901-14.

74. ACC/AHA guidelines for the management of patients with valvular heart disease. A report of the American College of Cardiology/American Heart Association. Task Force on Practice Guidelines (Committee on Management of Patients with Valvular Heart Disease). J Am Coll Cardiol, 1998. 32(5): p. 1486-588.

75. Institute, S.N.S., Order 1016/2013 Official population figures resulting from the review of the national census to January 1, 2013. 2013(311): p. 4.

76. Carton, J.A., et al., [Infectious endocarditis of the native valve: its epidemiological profile and an analysis of its mortality between the years 1984 and 1993]. Med Clin (Barc), 1995. 104(13): p. 493-9.

77. Fernandez-Hidalgo, N., et al., Contemporary epidemiology and prognosis of health care-associated infective endocarditis. Clin Infect Dis, 2008. 47(10): p. 1287-97.

78. Miro, J.M., et al., Staphylococcus aureus native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. Clin Infect Dis, 2005. 41(4): p. 507-14.

79. Brugal, M.T., et al., Evaluating the impact of methadone maintenance programmes on mortality due to overdose and aids in a cohort of heroin users in Spain. Addiction, 2005. 100(7): p. 981-9.

80. Lahoz Rallo, B., et al., [Consensus on care for the users of the Maintenance Program with Methadone at the health centers of the province of Cadiz]. Aten Primaria, 2000. 25(6): p. 417-21.

81. Puigdollers, E., et al., [Methadone maintenance programs with supplementary services: a cost-effectiveness study]. Gac Sanit, 2003. 17(2): p. 123-30.

82. Ruz Franzi, I., F. Gonzalez-Saiz, and F. Ruiz Avila, [Drug dependent patients in the methadone maintenance program: evaluation in primary care of psychosocial and organic severity]. Aten Primaria, 1998. 21(6): p. 384-8.

83. Martin-Davila, P., et al., Analysis of mortality and risk factors associated with native valve endocarditis in drug users: the importance of vegetation size. Am Heart J, 2005. 150(5): p. 1099-106.

84. Lisby, G., E. Gutschik, and D.T. Durack, Molecular methods for diagnosis of infective endocarditis. Infect Dis Clin North Am, 2002. 16(2): p. 393-412, x.

85. Thuny, F., et al., Management of infective endocarditis: challenges and perspectives. Lancet, 2012. 379(9819): p. 965-75.

86. Chirouze, C., et al., Enterococcal endocarditis in the beginning of the 21st century: analysis from the International Collaboration on Endocarditis-Prospective Cohort Study. Clin Microbiol Infect, 2013.

87. Douglas, P.S., et al., ACCF/ASE/AHA/ASNC/HFSA/HRS/SCAI/SCCM/SCCT/SCMR 2011 Appropriate Use Criteria for Echocardiography. A Report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, American Society of Echocardiography, American Heart Association, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, Society of Critical Care Medicine, Society of Cardiovascular Computed Tomography, Society for Cardiovascular Magnetic Resonance American College of Chest Physicians. J Am Soc Echocardiogr, 2011. 24(3): p. 229-67.

88. Incani, A., et al., Staphylococcus aureus bacteraemia: evaluation of the role of transoesophageal echocardiography in identifying clinically unsuspected endocarditis. Eur J Clin Microbiol Infect Dis, 2013. 32(8): p. 1003-8.

89. Kaasch, A.J., et al., Use of a simple criteria set for guiding echocardiography in nosocomial Staphylococcus aureus bacteremia. Clin Infect Dis, 2011. 53(1): p. 1-9.

90. Khatib, R. and M. Sharma, Echocardiography is dispensable in uncomplicated Staphylococcus aureus bacteremia. Medicine (Baltimore), 2013. 92(3): p. 182-8.

91. McAdam, A.J., Discrepant Analysis: How Can We Test a Test? J Clin Microbiol, 2000. 38(6): p. 2027-9.

92. Sarrazin, J.-F., et al., Usefulness of Fluorine-18 Positron Emission Tomography/Computed Tomography for Identification of Cardiovascular Implantable Electronic Device Infections. Journal of the American College of Cardiology, 2012. 59(18): p. 1616-1625.

93. Sharara, A.I., et al., Association of Streptococcus bovis endocarditis and advanced colorectal neoplasia: A case-control study. J Dig Dis, 2013. 14(7): p. 382-7.

94. Thomsen, R.W., et al., Endocarditis and risk of cancer: a Danish nationwide cohort study. Am J Med, 2013. 126(1): p. 58-67.

95. Normativo, S.G.d.R.J.y.D. and C.d.C.y.D.y.P.d.G. S.G.T de Vicepresidencia, ORDEN POR LA QUE SE FIJAN LOS PRECIOS PÚBLICOS POR LA PRESTACIÓN DE LOS SERVICIOS Y ACTIVIDADES DE NATURALEZA SANITARIA DE LA RED DE CENTROS DE LA COMUNIDAD DE MADRID. 2009: Madrid. p. 29.

96. Gotthardt, M., et al., Imaging of Inflammation by PET, Conventional Scintigraphy, and Other Imaging Techniques and Other Imaging Techniques. J Nucl Med, 2010(51): p. 13.

ANNEXES